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THE CLINICAL USE OF SYNTHETIC MALE SEX HORMONE

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CLINICAL experience with the male sex hormones or androgens as substitute therapy for hypogonadism has received attention in only a few reports, and these mostly during the past year. With the introduction of purified and synthetic hormones, it becomes feasible to investigate with greater certainty the influence of the androgens on the genital structures. The purpose of this study is to present the results of two years' experience with synthetic testosterone propionate in the treatment of hypogenitalism, both prepuberal and adult types, impotency, symptoms of sexual decline, benign prostate hypertrophy, and gynecomastia.

This report is based on the observation of a group of 54 males ranging in age from 7 to 75 years, and showing various forms of genital hypoplasia and malfunctions of the sex organs. The material presented here summarizes the pertinent facts of 15 selected cases as typical of the group, which are distributed as follows: 11 presented hypogenitalism, 2 complained of impotency and symptoms of sexual decline, 1 exhibited well-developed symptoms of prostatic hypertrophy, and 1 had well-advanced gynecomastia. Each patient was subjected to a thorough physical and laboratory examination before the treatment was begun. The androgenic treatment consisted of intramuscular injections of testosterone propionate[1] given 2 or 3 times weekly in doses of from 10 to 25 mg. dissolved in 1 cc. sesame oil. The effectiveness of the treatment was determined in the hypogenital patients by observations on the generalized changes in sex characteristics which included voice change, growth of hair, increasing size of phallus, production of erections with emissions, development of libido and potentia, and in the prostatic hypertrophy case by clinical improvement.

[1] Generously supplied for this study by Dr. Max Gilbert of the Schering Corporation.

I am deeply grateful to Dr. A. J. Lehman, of the Department of Pharmacology, for his aid in the preparation of this report.

CASE STUDIES

Case 1. K. B., aged 11 years, had genital underdevelopment and bilateral cryptorchidism. The maldescent of the testes was successfully treated with APL hormones, but the testes and penis remained small. An attempt was made to increase penile growth with testosterone injections; 10 mg. 3 times weekly over a period of 4 weeks definitely increased the size of the penis.

Case 2. G. C., (fig. 2) a 40-year-old male, complained of his feminine appearance and also that he had no testicles. He had married at the age of 21, and occasional unsuccessful attempts were made at intercourse. His life had been quite unhappy because of sensitiveness to his condition. The patient presented the typical appearance of an obese hypogonad individual, with striking disproportion in the length of the extremities compared to the trunk. The obesity was confined to the trunk and included mammary fat paddings, pendulous abdomen, and mons veneris shelving. There was no beard on his face, and only a scant amount of hair over the pubes. The patient had a sallow complexion, with rather sharp, feminine-like features. The penis was about the size of that of a boy of 8 years. The scrotum was atrophic, and no testicles or prostate could be palpated. Roentgenograms showed the sella turcica within normal limits, and those of the extremities showed delay in epiphyseal closure. Findings of routine blood and urine examinations were normal. During 1933 and 1934 he received 19,200 U of antuitrin-S and prephysin, with some improvement in his general feeling of well-being, but with no appreciable objective changes (1).

Treatment consisted of 25 mg. of testosterone propionate 3 times weekly. After the 6th injection, he reported having erections and emissions whenever in the presence of the opposite sex. After the 12th injection, he had successful intercourse for the first time in his life. The sex urge increased and he continued to feel very energetic. His voice began changing to a deeper pitch, and it was obvious that the penis was growing. At the end of 13 weeks' treatment, he was a definitely changed individual. His weight was much better distributed, the penis had grown remarkably, and there was increased hair growth over the entire body. Libido, potentia coeundi, and sex functions in general continued to be almost normal, and his voice tone had lowered to a degree that his friends did not recognize him over the telephone. After receiving 1100 mg. of testosterone propionate over a period of 18 weeks, the treatment was abruptly changed to placebo injections of sesame oil and continued for 6 weeks. Toward the end of this time period, he began complaining of not feeling as well as before. Libido decreased and there was a loss of 7 lb. in weight. There was neither repression nor increase of hair growth. Turgescence and fullness of the scrotum decreased, and it became increasingly more difficult for him to carry on sexual intercourse. His weight fell to 226½ lb. from a previous 233½ lb. When injections of the testosterone were again instigated, subjective improvement was immediate. He has received a total of 2925 mg. to date, and his progress has continued satisfactorily. His weight is again 233½ lb.; increased hair growth has been noted. The sexual functions are essentially normal. A small prostate could now be felt, but the testes showed no change from the original condition. No spermatozoa were found in the ejaculate which measured 0.6 cc. The voice deepened remarkably, and the larynx grew to such an extent that it became necessary to increase the shirt size by one-half, even though there has been no increase in body weight. The x-ray picture did not change. Blood pressure remained normal at all times.

Case 3. P. S., (fig. 3) aged 26 years, complained of genital underdevelopment. He stated that he has always been somewhat taller than other boys of his age and that

Fig. 1. Case 18, Case 3, Case 4, Case 22, Case 2, Case 36. Hypogonadism—A group of various types and ages.

his voice has always remained rather high-pitched. He has never had any desire for the opposite sex and has never had an erection.

The physical examination revealed a typical non-obese hypogonad individual.

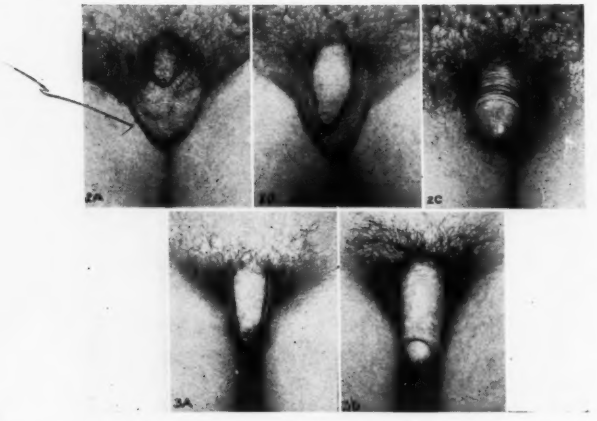

Fig. 2, Case 2, aged 40 years. A, before; B, after injection of 1110 mg. testosterone propionate; C, after 2925 mg. of testosterone propionate. Fig. 3. Case 3, aged 26 years. A, before; B, after injection of 1630 mg. of testosterone propionate.

There was no hair on the face or in the axillae, and only a few scattered hairs over the pubes. He has never shaved. The penis was approximately 4 cm. in length and 3.8 cm. in circumference. The scrotum was infantile, and the right testicle was not palpable. The findings of blood and urine examinations were essentially normal. Roentgenograms revealed a pituitary fossa of normal size and ununited distal epiphyses of the radii and ulnae which were comparable to those of an average male of

Fig. 4. Roentgenograms of Case 3 showing no advancement of epiphyseal union after 45 weeks androgenic therapy.

17 years. During a 6-month period in 1936, he received 11,500 R.U. of antuitrin-S, and during the latter third of this period, antuitrin-S plus antuitrin gonadotropic. There were no subjective nor objective signs of improvement as a result of this treatment, possibly due to inadequate dosage.

Treatment was begun with 10 mg. of testosterone propionate 3 times weekly. After the 4th injection, erections became frequent and there was increased sexual feeling which he had never experienced previously. He complained of some tenderness and swelling of the breasts. There were no emissions nor ejaculations, and no intercourse had been attempted. After 18 injections, there was a perceptible increase in the size of the penis and some evidence of scrotal development. The prostate, which could not be defined at the beginning of treatment, had now enlarged until both lobes were clearly outlined and estimated to be about the size of a shelled almond. Growth of hair increased over the pubes, but the face and axillae remained free. After 16 weeks of treatment, giving a total of 560 mg., he had gained 21 lb. in weight; his general appearance and demeanor were that of an average individual. Erections occurred daily, but the ejaculate, amounting to 0.2 cc. of white viscid fluid, contained no spermatozoa. The left testicle was about one-third larger than originally and had descended farther into the scrotum; the right was now palpable in the upper pole and had apparently increased in size. Pubic hair was increasing but no hair had as yet appeared on the face. Roentgenograms showed no progress in epiphyseal closure (fig. 4). Libido was experienced for the first time in his life. He has now been treated for 45 weeks and has received a total of 1630 mg. testosterone, with continued improvement in all secondary sex characteristics.

Case 4. G.M., (fig. 5) aged 29 years, presented genital hypoplasia. He stated that he had had infrequent erections and an occasional orgasm. The examination showed a very thin, undernourished male with the usual disproportion of measurements. There was no facial hair, and he stated that he had never shaved. There was very scant axillary and pubic hair. The penis was quite small. Both testes were descended and well developed. The prostate was small, thin and flat. Findings of blood and urine examinations were normal. Roentgenograms of the skull indicated a small sella turcica, and those of the wrists showed the epiphyseal lines still quite evident. Four years ago he received 1 cc. of plain antuitrin daily for approximately 3 months, without any appreciable change in his condition, and some time later antuitrin-S, 100 U every other day for a period of 2 months, which he believed to be of some benefit. Treatment was instituted with injections of testosterone propionate, 10 mg. 3 times weekly. Following the administration of 180 mg., the libido and potentia coeundi increased. Erections and ejaculations became more frequent. Hair on the pubes became more abundant, and the penis and testes increased in size. After 11 months of treatment, total dosage 3150 mg., the penis had tripled in size and the prostate assumed a fuller normal contour and enlarged to approximately one-half average size for the age. His sex urge and power increased remarkably. Hair on the face and pubes showed increased growth.

Case 5. H.M., (fig. 6) white male, aged 16 years, complained of obesity and underdevelopment of the genitals. Physical examination showed a typical adiposogenital individual with girdle obesity and rather extreme mons veneris padding. The testes were about normal in size, but apparently fixed in the extreme upper poles of the scrotum. The penis was infantile. A few hairs were scattered over the pubes. Results of routine laboratory examinations were essentially normal.

The first stage of treatment consisted of a prolonged course of injections of the APL hormones, with complete descent of the testes but without any evident increase

of the size of the penis. Injections of testosterone propionate, 10 mg. 3 times weekly, were then begun. After the 5th injection, he began having morning erections and occasional nocturnal emissions. After 4 weeks of treatment, increase in the size of the penis was noted and the scrotum was becoming the serrated, adult type. Conse-

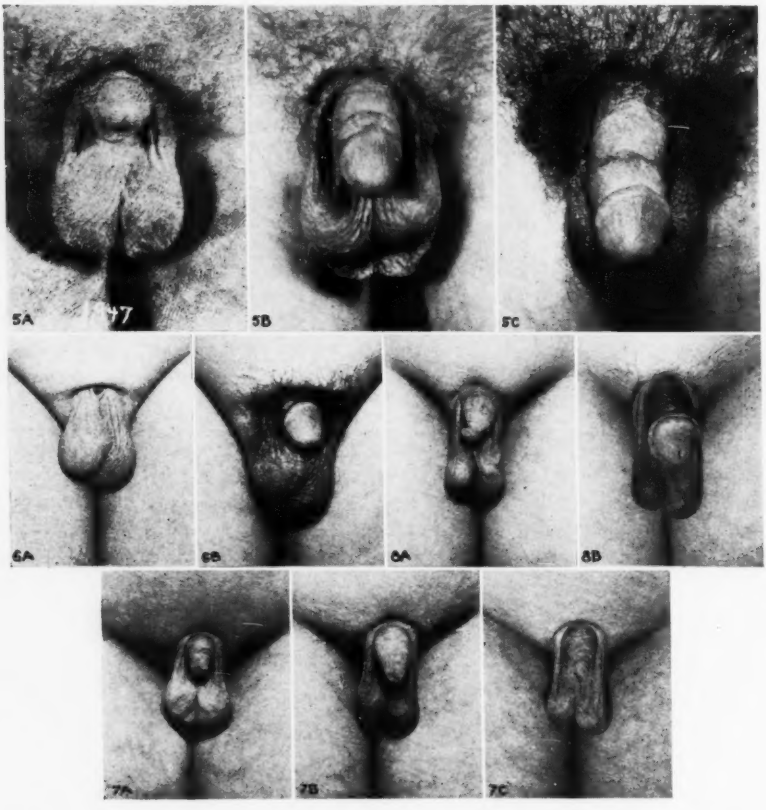

Fig. 5. Case 4, aged 20 years. A, before; B, after injection of 540 mg.; C, after 3150 mg. testosterone propionate. Fig. 6. Case 5, aged 16 years. A, testes descended and developed normally, but penis remained infantile after injection of 42,000 R.U. of APL; B, after injection of 330 mg. testosterone propionate. Fig. 7. Case 8, aged 9 years. A, before; B, after treatment with synthetic male sex hormone; C, slight regression 10 weeks after cessation of therapy. Fig. 8. Case 11, aged 12 years. A, before; B, after 10 weeks treatment.

quently, the dosage was reduced to 10 mg. twice weekly. The penis continued to increase in size, and pubic hair became more abundant. He has now received a total of 330 mg. in 3 months, with continued improvement. The voice has deepened, and the general body contour is more masculine. No facial or axillary hair has appeared as yet. Erections and ejaculations are of frequent occurrence.

Case 6. C.C., aged 9 years, was a poorly developed and undernourished boy with bilateral cryptorchidism. The scrotum and penis were underdeveloped. The initial treatment consisted of APL hormone injections which were quite successful in bring-

ing the testes into position. The penis and scrotum, however, remained underdeveloped. Testosterone propionate was then started, 10 mg. being given twice weekly. After the 6th injection, he began having erections 2 and 3 times weekly. The penis began growing quite rapidly, and a few scant pubic hairs appeared. The dosage was increased to 25 mg. twice weekly, and after the 3rd injection he complained of almost constant priapism. After a 2-week rest period, during which time no treatment was given, the original dosage was again instituted for 14 injections, then discontinued. The penis showed normal growth. Four months later the genitalia appeared normal for his age, with the exception of the left testis which remained small and atrophic.

Case 7. F.G., aged 12 years, was a typical adiposogenital type, who had progressed satisfactorily during a period of AP gonadotropic therapy, but was given testosterone propionate, 10 mg. twice weekly, to demonstrate its rapidity of action. At the end of 4 weeks of treatment, there was definite increase in the size of the penis, scrotum, and testes. Frequent erections were experienced, and ejaculations upon manipulation.

Case 8. B.R., white male, aged 9, presented a typical Fröhlich's syndrome. Both testes were descended, but the penis was quite small and underdeveloped. Roentgenograms of the wrists and sella showed nothing abnormal; the blood and urine were normal. The early stages of the treatment were directed toward combating the obesity. The usual measures were employed, and within 3 months the boy's weight was reduced sufficiently to bring him within the average weight limits for his age. Injections of AP gonadotropic hormone, 400 U weekly, were given for a period of 3 weeks. No development of the penis occurred. Testosterone injections were begun in 10 mg. doses twice weekly. After the 13th injection, the penis was definitely growing and erections as frequently as 3 to 4 times weekly were reported. Treatment was stopped after the 25th injection. Penis growth was much slower than expected, and repeated examinations during the past 4 months show that the penis is regressing, as shown in figure 7.

Case 9. G.S., a 7-year-old white male with hypogenitalism, was first seen 3 years ago. Physical examination at that time revealed a small right testis just below Poupart's ligament with the left not palpable either in the scrotum or canal. He was chubby but not obese. Following the injection of a total of 3400 U of antuitrin-S over a period of 13 weeks, the right testis had assumed a position in the upper part of the scrotum, but the left was still not palpable. The penis showed no evidence of growth.

The patient was not seen during an interim of 27 months. Examination at this time disclosed that the left testis had not descended; the right descended but underdeveloped; the penis still infantile. Androgenic hormone injections in doses of 5 mg. twice weekly were begun. After 8 injections, increase in the size of the penis was noted, and after 16 injections (total 80 mg.), a remarkable growth in the genitalia was observed and erections were frequently experienced. There was no change in the size or position of the testes.

Case 10. S.B. was a 9-year-old boy with adiposogenitalism and non-descent of the testes. The penis was slightly underdeveloped and the scrotum definitely so. The initial treatment consisted of APL hormone administrations, a total of 3600 U being given over a period of 16 weeks. Some evidence of testicular descent was observed, as well as some increase in the size of the penis, but the genitalia were still underdeveloped. The hormone injections were continued until 18,700 U were administered over a period of 9 months. At this time, the testes were in proper position and the penis and testes bordered on the lower limit of normal for the age. Ten mg. of testosterone administered twice weekly for one month only promoted the development

of the penis to average size. All injections have been discontinued. Repeated examinations over the past 6 months show that the genitalia are developing normally. However, no erections or ejaculations have been reported.

Case 11. M.R., (fig. 8) a male, aged 12 years, complained of overweight and enuresis. The patient presented a classical adiposogenitalism with mammary padding and abdominal folds. Both testes were descended although underdeveloped. Findings of routine laboratory examinations were essentially normal. The treatment with testosterone propionate, 10 mg. 2 times weekly for 13 weeks, promoted development of the genitalia. The penis increased in size and thickness, and occasional erections and ejaculations were experienced. The testes showed no further growth. The enuresis decreased in frequency, and for a period of 2 weeks there was no bed wetting. At present, 5 mg. of the androgen is being given twice weekly. The enuresis is improved, although not entirely controlled. There are occasional erections and ejaculations, and the genitalia are showing continued development.

Case 12. C.B.S., aged 56 years, presented symptoms of sexual decline, including nervousness, irritability, and increasing fatigue. He also complained of prostatic pain, nocturia, and had been impotent for the past 3 years. Physical examination revealed no pertinent deviations from the normal for a man of his age. Treatment was begun with injections of testosterone propionate, 5 mg. twice weekly. After the 3rd injection, considerable improvement in his general well-being was noted. After 10 injections, sexual intercourse could be carried out satisfactorily. Upon substitution of the base oil for the testosterone, symptoms returned but promptly disappeared when the injections were resumed. This replacement therapy has been continued for a period of 19 months and has kept him sexually normal up to the time of this writing.

Case 13. H.A.N., aged 48 years, complained of increasing impotency, premature ejaculations, and nocturia 3 times nightly. In addition to the above, he noticed that during the past year there has been increasing fatigability, nervousness, irritability, and some hesitancy in starting and loss in force in the urinary stream. Warm flashes occurred from time to time. The prostate was smooth in outline, slightly tender, and enlarged approximately one and one-half times.

Ten mg. of testosterone propionate twice weekly greatly improved his condition. At the end of 3 weeks of treatment he felt much stronger, less nervous, and had a general sense of well-being. The urinary flow was also much freer and stronger. The prostate seemed slightly reduced in size and less tender to palpation. After improvement occurred, the dose was reduced to 5 mg. twice weekly, which has been continued for the past 6 months. The beneficial effects have been maintained with this dosage.

Case 14. B.E., aged 75 years, had a simple benign prostatic hypertrophy. This patient presented the usual signs of partial prostatic obstruction. Ten mg. of testosterone were injected 3 times weekly for a period of 5 weeks. Complete relief of the frequency and other disturbances of micturition were obtained. Examination of the prostate suggested less turgescence and some reduction in size.

Case 15. J.C.B., (fig. 9), 23-year-old white male, complained of overdevelopment of his breasts. This overdevelopment began at the age of 13, when they became quite full and of the female type. There has been no change in size during the last 6 years. He has had a normal sex life, but the breasts were a source of embarrassment and he wanted them reduced.

The patient was tall, thin-faced, with a sallow complexion. The general body build was quite feminine. The breasts were full, rounded, well developed, virginal, with glandular tissue and well-formed nipples. The hair growth over the entire body

was somewhat increased. The penis was approximately normal, and the testes well descended and normal in size. All laboratory work yielded essentially normal results. A specimen of ejaculate measured 3.2 cc., with a total count of 8,000,000 active spermatozoa per cc. The blood cytology, serology and chemistry were all within normal limits. Assay for estrogenic hormone in a 24-hour urine specimen revealed approximately 15 R.U. (between 10 and 20 U is average for estrin assays in males). Androgenic hormone assay of the same specimen showed 28.6 I.U., using continuous benzol extraction and Koch's method (normal average between 20 and 80 U).

Fig. 9. Case 15, aged 23 years, with normal genital development. No reduction in size of breasts after the injection of 650 mg. of testosterone propionate.

Testosterone propionate in 25 mg. doses twice weekly was given for the past 13 weeks in an attempt to reduce the breasts. More frequent erections were experienced, and the patient felt better physically, but no appreciable change in the size of the breasts has occurred to date.

COMMENT

The observed favorable clinical responses to testosterone propionate are in agreement with the reports of Kenyon (2), Walther (3), Vest (4), and Hamilton (5). The body changes were limited to increase in hair growth, enlargement of the larynx, and a better distribution of weight. The appearance of pubic hair was observed in all cases, and in some there was growth over other parts of the body, including the extremities, axillae, and face, to a greater or lesser degree. Deepening of the voice and enlargement of the

larynx occurred in those cases past the usual age of adolescence. In one instance the larynx developed to such an extent that it became necessary for the patient to increase the shirt size by one-half, despite the fact that there had been a loss in weight. Changes in body contour were quite evident, especially in the adiposogenital cases. In the obese type a gradual disappearance of the abdominal and hip fat pads occurred with a better distribution of weight and the development of more masculine features. In thin individuals a distinct weight gain was noted; in one case an increase of 21 lb.

Bone changes could not be demonstrated. Roentgenograms in all of the true hypogonad cases showed a marked delay in epiphyseal closure; no evident progress in epiphyseal advancement was noted even after prolonged treatment with testosterone.

Genital development was one of the most constant features. Material enlargement of the penis was seen in all patients. Concomitant with penile growth, erections occurred more or less frequently and ejaculations were reported in the older cases. Spermatogenesis was not observed in any case, although in two patients under treatment the testes were normal in size and well descended into the scrotum. There was evidence of testicular growth in some patients; also, there was an increase in the size of the scrotum and a tendency to assume a more adult type.

Definite evidence of prostatic growth was also observed. In the hypogonad individuals the prostate could not be palpated, but after treatment with testosterone, the gland could be easily felt. In no case, however, has the growth kept pace with normal for the age. In prostatic hypertrophy, there appeared to be some decrease in size, to manual palpation, along with definite improvement of all obstructive symptoms—urgency, frequency, and nocturia. Noteworthy improvement was also achieved in 2 patients with enuresis. In one instance complete relief for 2 weeks was obtained, although there was a recurrence later in a much milder degree.

Changes in the breasts were not observed. However, some transient swelling and soreness was complained of in 3 instances. In the single case of gynecomastia reported here, testosterone failed to bring about any regression in the size. This is in agreement with other reports of gynecomastia with normal genital development (6).

Augmentation of the sexual powers was experienced by all patients. In the older hypogonad individuals the increased libido and potentia coeundi enabled them to carry out the sexual act satisfactorily for the first time in their lives. The symptoms associated with sexual diminution associated with senescence, such as nervousness, irritability, insomnia, increasing fatigue, apprehension, and gastro- intestinal disturbances, all responded to treatment.

Much was also accomplished with respect to the mental attitude of the patients. Practically every hypogonad individual had to a greater or lesser extent, a sense of inferiority, but as treatment progressed and sexual manifestation increased, there was a feeling of buoyancy, increased physical and mental activity, and the development of a more aggressive masculine type of personality.

Blood pressure changes in all patients treated were of little significance. Readings were made repeatedly throughout the course of the treatment, and in no instance were there any fluctuations greater than the normal variations. However, no hypertensive cases are included in this report.

Despite the fact that no undesirable actions have been observed in any cases treated with testosterone propionate, both the untoward as well as helpful effects should be looked for carefully and critically, and the decision as to the continuance of treatment guided by the results.

SUMMARY

Fifty-four patients with various types of genital anomalies and dysfunctions, which included adult hypogonadism, sexual diminution associated with senescence, adiposogenitalism, gynecomastia, and benign prostatic hypertrophy, were treated with the synthetic male sex hormone, testosterone propionate. In doses of 10 to 75 mg. weekly, it was found effective in every hypogonad case, as evidenced by promoting hair and penile growth, producing erections and emissions, developing the libido and potentia coeundi, and by generalized changes in secondary sex characteristics. Gynecomastia with normal secondary sex development is apparently not influenced by injections of the hormone. The androgen brought about considerable relief of the subjective symptoms of sexual diminution associated with senescence, and in cases of benign prostatic hypertrophy, as shown by the clinical improvement in the usual symptoms and signs of prostatic obstruction. No untoward by-effects were noted in any of the cases treated.

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THE TREATMENT OF ADDISON'S DISEASE WITH ADRENAL CORTEX EXTRACT[1]

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IN MANY STUDIES on the treatment of Addison's disease the administration of an adrenal cortex extract has been combined with the administration of sodium salts, and it is frequently difficult or impossible to determine which produced the beneficial effects. The object of this report is to demonstrate the value of the extract itself. During the past three and one-half years we have made observations on treatment with an adrenal cortex extract (1, 2, 3), with and without other forms of therapy. So far as we are aware, our observations on treatment with the extract alone cover a much longer period of time than any in the literature and are now extensive enough to prove that it possesses some potency. It can not be emphasized too strongly that it is impossible to draw conclusions about the efficacy of any form of treatment from short periods of study.

We have used the adrenal cortex extract of the Wilson Laboratories,[2] each cubic centimeter of which represents 75 grams of fresh beef adrenal. This material is water-soluble and does not cause any reaction, except that occasional lots produce transient palpitation and nervousness, which suggest the presence of a trace of epinephrine. For maintenance therapy it is injected subcutaneously and in crises, intravenously. Recent reports by Grollman and Firor (4) and Thorn et al. (5) indicate that cortical extracts may be of some value when given by mouth. However, we have not found it as effective by this route as have these observers.

The effect of prolonged administration of the extract has been observed in 7 patients with marked Addison's disease, varying in age from 18 to 49 years. All of them showed the typical clinical findings of the disease, including pigmentation, reduction in blood pressure and characteristic chemical alterations in the blood. In 2 patients active tuberculosis was present. Of the 7 patients, 4 are now living and 3 are dead.

DESCRIPTION OF CASES AND RESULTS

Patients Now Living

The 4 patients who are still living have received the extract for from 1 to 3¼ years. All carry on their usual activities except one (*Mrs. D. R.*), who

[1] Read before the Twenty-second Annual Meeting of the Association for the Study of Internal Secretions, San Francisco, June 14, 1938.

[2] The extract was kindly supplied by Dr. David Klein.

is confined to the hospital because of active tuberculosis. Brief case histories of these patients are given below.

Patient 1. The first patient to receive the extract was *Mrs. R. Y.*, (fig. 1, 3) a Bohemian woman, who entered the Cook County Hospital Feb. 20, 1935 at the age of 46 years. She was admitted on the service of Dr. L. C. Gatewood who referred her to us. She complained of weakness, increased pigmentation of the skin and loss of 60 lb. over a period of 2 years. Apparently she survived a crisis 6 months before admission but remained in poor health. Three weeks before admission vomiting recurred. She showed marked emaciation, generalized brown pigmentation of the skin and pronounced pigmented patches on the mucous membranes of the gums, cheeks and hard palate and on the inner surfaces of the labia minora. Her systolic blood pressure was

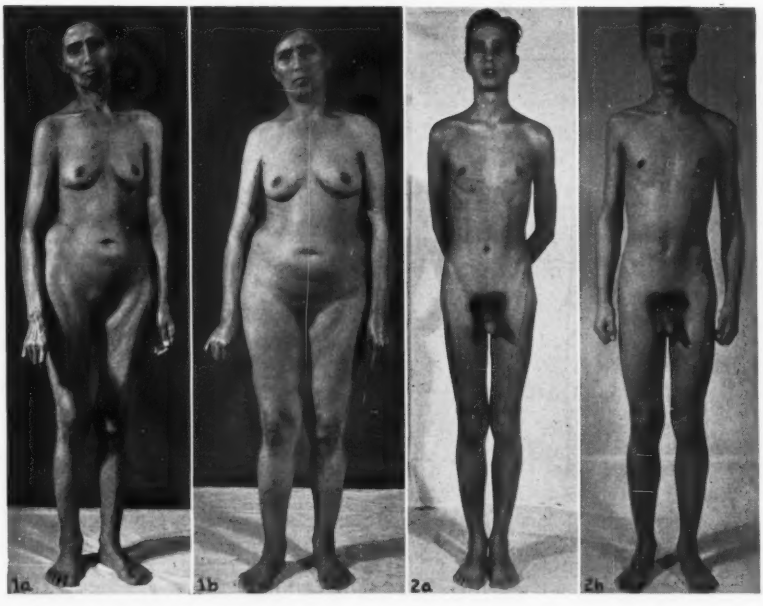

Fig. 1. *Mrs. R. Y.* (*patient 1*). Ht. 61 in. *a.* 5/7/35. Weight 86 pounds. By this time she had gained about 10 pounds. *b.* 4/6/36. Weight 117 pounds. Fig. 2. *Mr. F. K.* (*patient 2*). Ht. 70¾ in. *a.* 10/23/35. Weight 121½ pounds. *b.* 2/13/37. Weight 142½ pounds.

96 mm. Hg and her diastolic 60 mm. Hg. The hemoglobin was 60% and the red blood cell count was 2,470,000 per cu. mm. Shortly after admission she developed a severe follicular tonsillitis which was followed by lobar pneumonia in the lower half of the left lung. The patient became extremely ill, her blood pressure dropped to 80/56, her temperature rose to 106° F., and she was disoriented. Within a period of 10 days following the onset of pneumonia, 14,500 cc. of normal salt solution and 2000 cc. of 5% glucose were administered subcutaneously, and adrenal cortex extract was given intravenously in doses of from 10 to 37 cc. daily. She also received 3 gm. of sodium citrate and 12 drams of an iron preparation (Chapell's Hemo-S) daily by mouth. With this treatment and good nursing care, she gradually recovered. The total serum base rose from 142.7 to 155.8 m.eq. per liter (6). During convalescence

she received 20 cc. of the extract daily and the sodium citrate and iron were continued by mouth. On this regimen she improved markedly. On July 24th, the administration of 4 gm. of sodium chloride daily by mouth was started. The dose of extract had been gradually reduced and at the time of discharge from the hospital, Aug. 19, 1935, was 5 cc. daily. The sodium citrate and iron were omitted at this time. Sodium chloride was omitted and the dose of extract was increased to 10 cc. daily on Oct. 14th. Soon after discharge she was able to resume her normal duties at home. Since we first saw her, she has received the extract continuously except for two periods, one of 5 days and the other of 41 days duration. For the most part she has received 10 cc. daily, administered subcutaneously in 2 doses of 5 cc. each. For periods of from 27 days to 14 months this was the only form of therapy employed. She has had relapses only on the following occasions.

a) Seven days after the dose of extract was reduced from 10 cc. to 5 cc. daily in spite of the additional administration of 3 gm. of sodium citrate daily. This was about 2½ months after her recovery from pneumonia, while she was still in the hospital and weighed 88 lb. Later, at home, she did not develop symptoms when 5 cc. of extract and 4 gm. of sodium chloride were administered daily for 8 weeks. During this period her weight was about 98 lb.

b) On 5 occasions when she developed a mild or moderately severe upper respiratory infection (2/13/36, 4/17/36, 5/2/36, 4/11/37 and 1/13/38). On the last 4 dates she reported at once and the symptoms of Addison's disease cleared up promptly after the dose of extract was temporarily increased to between 15 cc. and 45 cc. daily. On the first occasion (2/15/36), no increase was made in the dose of extract but it was supplemented for a short time with the administration of sodium chloride and sodium bicarbonate. With one other mild upper respiratory infection (2/17/37) there were no symptoms of adrenal insufficiency, although, as a precaution, the dose of extract was increased from 10 cc. to 15 cc. daily for 2 days.

c) On two occasions when the extract was omitted. Because of the importance of the time relationships, the clinical courses during the two periods of omission of extract are recorded in detail.

October 26th, 1935, in order to observe whether the patient still had Addison's disease, the extract was omitted after she had received her regular dose of 10 cc. This was the only medication she was receiving at the time. On Oct. 29th her appetite was poor, on Oct. 30th she was nauseated all day and by Oct. 31st weakness had set in. Nausea continued and at 9:15 P.M. one emesis occurred. Her weight dropped from 101 lb. to 99½ lb. during this period, and her blood pressure from 114/66 to 94/78. The total serum base dropped from 150.7 (10/7/35) to 148.7 (10/31/35) m.eq. per liter. She was revived by the administration of 20 cc. of extract daily for 4 days and thereafter her usual dose of 10 cc. daily was sufficient.

During the second period of omission a much longer interval elapsed before symptoms developed. The omission was necessary because of temporary difficulty in securing the patient's cooperation. She omitted the extract for 41 days beginning Dec. 7th, 1936, except as recorded below. At the time of omission she weighed 120¾ pounds and her blood pressure was 134/100. From May 9, 1936 until the time of omission of extract she had been taking 3.6 gm. of sodium bicarbonate daily in addition to the extract. The sodium bicarbonate was continued until Dec. 13th, at which time her husband reported that there was no change in her condition. By Dec. 21st she noticed weakness, nausea, belching of gas and a decrease in appetite. These symptoms continued until extract was resumed. From Jan. 2, to 12, 1937 another physician administered 1 cc. of Wilson's extract daily by the subcutaneous

route. At the same time the patient took 6 capsules of Wilson's dried suprarenal gland by mouth daily and continued the dried gland therapy until a short time after we ourselves resumed the administration of extract. By Jan. 15 her weight had dropped to 111½ lb. and her blood pressure to 104/78. However, she was able to come to the hospital accompanied by her husband. On that day she was given 1.2 gm. of sodium bicarbonate by mouth and the next day 2.4 gm. was administered by mouth. It was then omitted. On Jan. 16 she felt weaker, although she did some of her shopping and prepared her husband's evening meal. She became nauseated about 10 P.M., but at no time during this period of omission of extract did vomiting occur. On Jan. 17, because of increased weakness, it was necessary for her to return to bed shortly after arising. She ate very little food, experienced some 'heartburn' and remained in bed most of the day. In the afternoon one of us (W.O.T.) was requested to see her at home. On arriving there at 4 P.M. her blood pressure was found to be 102/74 and her pulse rate 87. The pigmentation of her skin and on the inside of her right cheek was definitely increased. She complained of marked weakness. A specimen of blood showed the concentration of total base in serum to be 150.4 m.eq. per liter. During the next half hour she was given 30 cc. of extract intravenously in 3 doses of 10 cc. each. After the first injection she said she was thirsty and drank 125 cc. of 20% cream. After the second injection she was definitely brighter and by 4:45 P.M. she was walking around the house and said she felt well enough to go to the movies. This striking improvement occurred with very little change in her blood pressure, which was 100/74 at 4:43 P.M. An additional 20 cc. of extract was administered subcutaneously in divided doses on Jan. 17. On Jan. 18 she received a total of 25 cc. of extract and thereafter 15 cc. daily until Feb. 6, when the dose of 10 cc. daily was resumed. Her blood pressure rose slowly, being 106/78 on Jan. 18, 108/74 on Jan. 29 and 120/78 on Feb. 15. The dose of 10 cc. of extract daily was continued as the only form of treatment until April 22, 1938, when oral administration was substituted for subcutaneous administration for purposes of clinical trial. On the dose of 10 cc. daily by injection, her weight gradually rose until it was 120 lb. on April 23, 1937: her skin became lighter, and she was completely free from symptoms of Addison's disease.

This patient's recovery with large doses of extract alone from crises of Addison's disease which developed after periods of omission of the extract and in association with upper respiratory infections, furnishes proof of its potency.

The length of time required for marked symptoms to develop during the second period of omission of extract, as compared with the first period, is of interest and may perhaps bear some relation to the striking improvement in her general condition before omission. She had gained a total of 45 lb. At the time of the first omission, she weighed 20 lb. less.

Her clinical condition was no better when she was taking 3.6 gm. of sodium bicarbonate daily in addition to 10 cc. of extract (May to Dec. 1936) than when she was taking only the 10 cc. of extract. Moreover, her condition was better when she was taking 10 cc. of extract alone than when she was taking 5 cc. of extract and 4 gm. of sodium chloride daily in addition to that contained in her food (Aug. to Oct. 1935). Apparently, when an adequate dose of extract was administered, the addition of sodium salts was not of value.

On Sept. 24, 1935 she had her first menstrual period in 9 months. Since then she has menstruated a total of only 12 times, on the following dates: 11/10/35, 12/12/35, 1/25/36, 4/25/36, 7/1/36, 7/28/36, 8/29/36/ 9/29/36, 11/25/36, 4/6/37, 12/26/37 and 5/21/38. The flow has been scanty and lasted only one or two days. The prolonged intermenstrual periods and the scantiness of the flow may be attributed to the onset of the menopause or to Addison's disease, or to both factors. In contrast to *patient 3* described below, at no time was there any aggravation of symptoms of Addison's disease preceding menstruation.

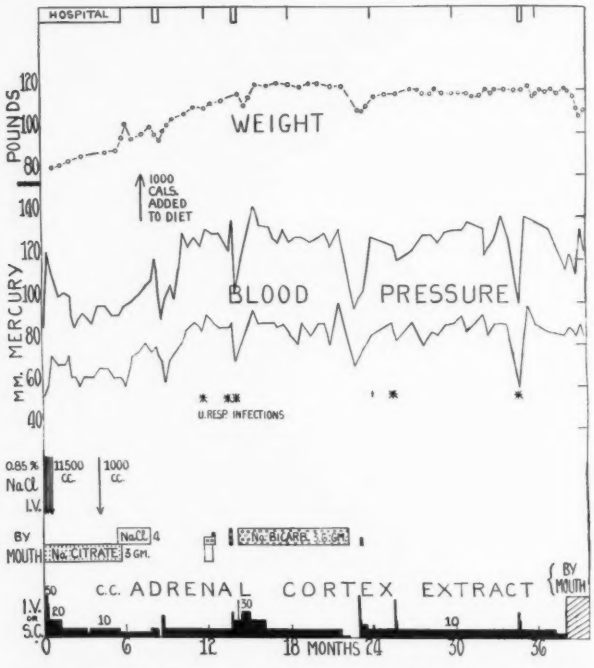

Fig. 3. Summary of treatment and clinical course of *Mrs. R. Y. (patient 1).*

During treatment her weight increased from about 75 lb. to 122 lb. and the secondary anemia present in the beginning disappeared. At the time of her first photograph taken in May 1935 (fig. 1) she had gained about 10 lb. Her gain in weight was aided by the administration of 1000 extra calories daily in the form of 20% cream given between meals. This was well tolerated and produced no gastro-intestinal disturbance in spite of the difficulty reported to be experienced by patients with Addison's disease in digesting fat. During periods of well being, after her first discharge from the hospital, the total serum base varied from 150.7 to 154.0 m.eq. per liter; and during crises, from 148.8 to 150.4 m.eq. per liter. Three basal metabolism determinations from Aug. 21st, 1936 to Dec. 7th, 1936 varied from -6 to -9%. The

pigmentation of her buccal mucous membrane and of her skin decreased markedly but did not disappear completely. However, in her mouth, many small areas of pigmentation actually did disappear. The relation between the level of blood pressure and body weight (fig. 3) is discussed below.

Since leaving the Cook County Hospital in Aug. 1935, this patient has done all her own housework except during periods of omission of the extract and during upper respiratory infections. She has appeared alert and has not complained of unusual fatigue. Her activity has been restricted very little by the presence of Addison's disease. From her appearance at the present time, the condition would not be suspected.

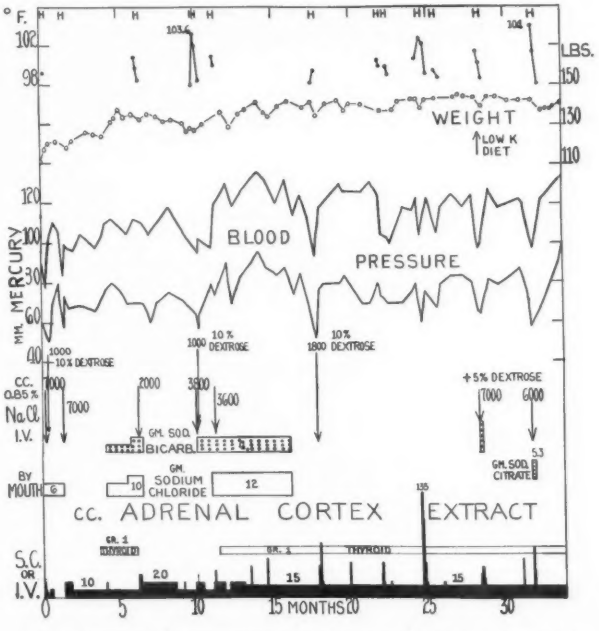

Fig. 4. Summary of treatment and clinical course of *Mr. F. K. (patient 2).* "H" denotes periods in the hospital.

Patient 2, Mr. F. K., (figs. 2, 4), an American business school student, has been under our observation since Oct. 7, 1935 when, at the age of 18 years, he was referred to us by Dr. J. Roscoe Harry. He had been admitted to the hospital in a crisis. The day before, he had lost his appetite and developed dull epigastric pain. He vomited his breakfast on Oct. 7th, fainted during the morning, and was then taken to the hospital in a state of complete exhaustion. He was very weak and lethargic. His temperature was not elevated. The systolic blood pressure was 90 and the diastolic, 60 mm. Hg. His skin was cold and clammy and showed a generalized dark brown pigmentation. He had had symptoms of Addison's disease since April 1934. Before being referred to us he had had 5 crises and his treatment had consisted of the administration of as much as 6 gm. of sodium chloride daily, thyroid extract and small

TABLE 1. SUMMARY OF CRISES IN *F. K.*

Date of crisis	DAILY MAINTENANCE THERAPY AT TIME CRISIS DEVELOPED						CRISIS	
	Adrenal cortex extract subcutaneously	Desiccated thyroid by mouth	NaCl by mouth	Sodium bicarbonate by mouth	Diet	Insulin U	Length of time in hospital	Medication
10/ 7/35	cc. Armour's 2	gr. intermittently 1/2	gm. 6	gm. —	High protein	—	10/7–10/12	Normal salt solution intravenously, 5000 cc. 10/7–10/9. 10% dextrose intravenously, 1000 cc. 10/7. Armour's adrenal cortex extract intravenously, 6 cc. (2×3) 10/7. Wilson's adrenal cortex extract intravenously, 20 cc. (10×2) 10/8; then 10 cc. (5×2) daily. Sodium chloride by mouth, 6 gm. daily continued.
10/13/35	Wilson's 5	—	6	—	General	—	10/13–10/15	Normal salt solution intravenously, 2000 cc. 10/13. Wilson's adrenal cortex extract intravenously 5 cc. daily. Sodium chloride by mouth, 6 gm. daily continued.
11/17/35	none since 10/23	—	6	—	General		11/18–11/20	Normal salt solution intravenously, 7000 cc. 11/18–11/19. Wilson's adrenal cortex extract intravenously, 20–30 cc. (10×2 or 3) daily. Sodium chloride by mouth, 8 gm. daily 11/18–11/19. Then omitted. Sodium bicarbonate by mouth, 4 gm. daily, 11/18–11/20. Then omitted
2/10/36	Wilson's 10	1	—	—	High caloric	—	0	Wilson's adrenal cortex extract intravenously, 20 cc. (10×2) on 2/10; then 10 cc. daily. Sodium chloride by mouth, 6 gm. daily started 2/11. Sodium bicarbonate by mouth, 2 gm. daily started 2/11.
3/17/36	10	1	6	2	High caloric	10	0	Wilson's adrenal cortex extract intravenously, 20 cc. (5×4) daily 3/19–3/22. Sodium chloride by mouth increased to 10 gm. daily 3/17. Sodium bicarbonate by mouth, 2 gm. daily continued.
4/12/36	10	1	10	4	High caloric	—	4/12–4/16	Normal salt solution intravenously, 2000 cc. 4/12–13. Wilson's adrenal cortex extract intravenously, 30 cc. (5×6) daily, 4/12–4/15: 20 cc. (5×4), 4/16. Sodium chloride by mouth, 10 gm. daily continued. Sodium bicarbonate by mouth, 4 gm. daily continued.
8/ 3/36	10	—	—	—	High caloric	—	8/3–8/8	Normal salt solution intravenously, 3800 cc., 8/3–8/4. 10% dextrose in normal salt solution intravenously, 1000 cc. 8/4. Wilson's adrenal cortex extract intravenously, 20 cc. (5×4) daily 8/3–8/8. Sodium carbonate by mouth, 8 to 4 gm. daily, 8/4–8/8.
9/ 9/36	10	—	—	4.6	High caloric	—	9/9–9/17	Normal salt solution intravenously, 4200 cc. 9/9–9/11. Wilson's adrenal cortex extract intravenously, 20 cc. (5×4), 9/9; 35 cc. (10×3 and 5×1) 9/10; 20 cc. daily (5×4) 9/11–9/17. Sodium chloride by mouth 12 gm. daily 9/11–9/15. Sodium bicarbonate by mouth, 4.6 gm. daily continued.
10/ 9/36	10	1	12	4.6	High caloric	—	10/9–10/13	Wilson's adrenal cortex extract intravenously, 20 cc. (5×4) daily. Sodium chloride by mouth, 12 gm. daily continued. Sodium bicarbonate by mouth, 4.6 gm. daily continued.
11/16/36	15	1	12	4.6	High caloric	—	0	Wilson's adrenal cortex extract intravenously, 40 cc. (10×4) 11/16. Sodium chloride by mouth, 12 gm. daily continued. Sodium bicarbonate by mouth, 4.6 gm. daily continued.
12/18/36	15	1	12	4.6	High caloric	—	0	Wilson's adrenal cortex extract intravenously, 65 cc. (10×5: 5×3) 12/18. Sodium chloride by mouth, 12 gm. daily continued. Sodium bicarbonate by mouth, 4.6 gm. daily continued.
3/24/37	15	1	—	—	High caloric	—	3/24–3/27	10% dextrose intravenously, 1800 cc. 3/25. Wilson's adrenal cortex extract intravenously, 50 cc. (10×4: 5×2) 3/24: 70 cc (10×7) 3/25: 20 cc. (10×2) 3/26: 20 cc. (5×4) 3/27.
5/22/37	15	1	—	—	High caloric	—	0	Wilson's adrenal cortex extract intravenously, 45 cc. (10×3: 5×3) 5/22.
8/ 2/37	15	1	—	—	High caloric	—	8/2–8/4	Wilson's adrenal cortex extract intravenously, 30 cc. (5×6) 8/2: 45 cc. (5×9) 8/3: 20 cc. (5×4) 8/4.

TABLE 1—Continued

Date of crisis	DAILY MAINTENANCE THERAPY AT TIME CRISIS DEVELOPED						CRISIS	
	Adrenal cortex extract subcutaneously	Desiccated thyroid by mouth	NaCl by mouth	Sodium bicarbonate by mouth	Diet	Insulin U	Length of time in hospital	Medication
	cc.	gr.	gm.	gm.				
8/18/37	15	1	—	—	High caloric	—	8/18–8/20	Wilson's adrenal cortex extract intravenously, 30 cc. (5×6) 8/18: 20 cc. (5×4) 8/19: 15 cc. (5×3) 8/20.
10/21/37	15	1	—	—	High caloric	—	10/21–10/28	Wilson's adrenal cortex extract intravenously, 135 cc. (10×13: 5×1) 10/21: 100 cc. (10×10) 10/22: 50 cc. (10×5) 10/23: 45 cc. (10×3: 5×3) 10/24: 20 cc. (5×4) 10/25–10/28.
11/23/37	15	1	—	—	High caloric	—	11/23–11/28	Wilson's adrenal cortex extract intravenously, 20 cc. (5×4), 11/23–11/28.
2/15/38	15	1	—	—	High caloric	—	2/15–2/25	5% dextrose in normal salt solution intravenously, 7000 cc. 2/15–2/18. Wilson's adrenal cortex extract intravenously, 30 cc. (5×6) 2/15: 40 cc. (5×8) 2/16 and 17: 20 cc. (5×4) 2/18 and 19: 15 cc. (5×3) 2/20–2/25. Sodium bicarbonate by mouth, 8 gm. daily, 2/15–2/20: 5.3 gm. daily 2/20–2/25. Low potassium diet, 2/15–2/25.
5/ 6/38	15	1	—	—	High caloric and low potassium	—	0	Wilson's adrenal cortex extract intravenously, 50 cc. (5×10), 5/6.
5/27/38	15	1	—	—	High caloric and low potassium	—	5/27–6/3	5% dextrose in normal salt solution intravenously, 2000 cc. 5/27: 3000 cc. 5/28: 1000 cc. 5/29. Wilson's adrenal cortex extract intravenously, 65 cc. (5×13) 5/27: 50 cc. (5×10) 5/28: 30 cc. (5×6) daily 5/29, 5/30 and 5/31: 20 cc. (5×4) daily 6/1 and 6/2: 15 cc. (5×3) 6/3. Sodium citrate by mouth, 5.3 gm. daily 5/27–5/29: 4 gm. daily 5/30–6/3.

doses of Parke Davis' and of Armour's adrenal cortex extract. At the time of his sixth crisis, Oct. 7, 1935 he was revived with Wilson's adrenal cortex extract and normal salt solution intravenously. There has been some variation in his treatment, but from Feb. 1937 to Feb. 1938 he received 15 cc. of extract (and 1 grain of desiccated thyroid) daily as the only form of therapy, except in crises.

Unlike *Mrs. R. Y.*, he has had repeated attacks of adrenal insufficiency, a total of 20 between Oct. 7, 1935 and May 27, 1938. These attacks were frequently ushered in by pain in the lower part of the chest and developed at intervals of 6 days to 16 weeks, usually with no obvious precipitating factor. Sometimes he lost a few pounds before nausea set in, but often felt well up to the day symptoms began. The relation of these attacks to the type of treatment is shown in table 1. In the intervals between attacks he usually felt well, had a good appetite, was able to attend business school, and to take part in various sports such as swimming, tennis and skiing.

It may be seen that he developed crises while taking the following forms of treatment:

6 gm. sodium chloride daily
10 cc. extract daily
15 cc. extract daily
10 cc. extract plus 4.6 gm. sodium bicarbonate daily
10 cc. extract plus 12 gm. sodium chloride and 4.6 gm. sodium bicarbonate daily
15 cc. extract plus 12 gm. sodium chloride and 4.6 gm. sodium bicarbonate daily
15 cc. extract daily plus a low potassium diet

If the crisis had not progressed very far, it could usually be aborted by administering from 30 to 40 cc. additional extract intravenously in the course of from 3 to 4 hours: but when the crisis was well advanced and nausea and vomiting were

marked, this had to be combined with the intravenous administration of normal salt solution.

From April 20, 1936 to June 24, 1936 he did not develop a crisis while receiving 20 cc. of extract daily without any other form of therapy. However, when the dose of extract was reduced to 10 cc. daily on June 24, 1936 he remained free from symptoms until August 3, 1936. Because of the comparatively long period of freedom

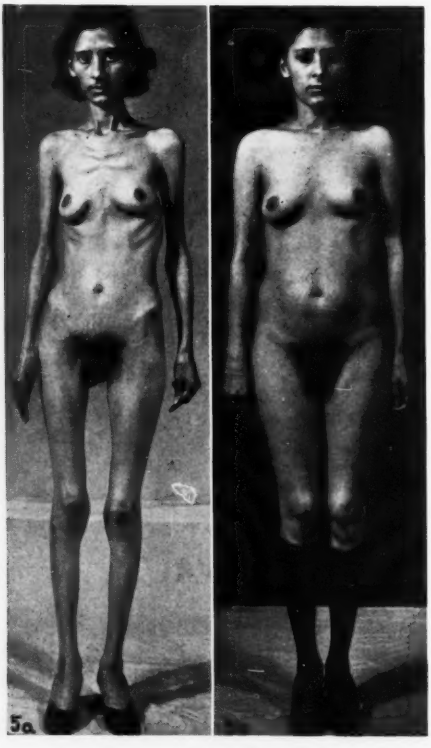

Fig. 5. *Mrs. D. R.* (*patient* 3). Ht. 63 in. *a.* 6/8/37. Weight 71½ pounds. By this time she had gained 6½ pounds. *b.* 9/16/37. Weight 103½ pounds.

from symptoms while he was receiving 10 cc. of extract daily as the only form of treatment, it can not be concluded that he would have remained symptom-free had the dose of 20 cc. daily been continued.

During treatment his weight has increased from 110 to 144 lb. the highest it has ever been. Just as in *Mrs. R. Y.*, his pigmentation has decreased but has not disappeared. His blood pressure has risen to normal. The total serum base has varied from 149.8 to 155.8 m.eq. per liter during periods of well-being, and from 143.6 to 154.7 m.eq. per liter during crises. The basal metabolism level was −16% and on Jan. 29, 1936, Wilson's desiccated thyroid in a dose of 1 grain daily was prescribed. This was omitted on April 11, but restarted Sept. 19, 1936 and continued thereafter. It increased his energy but did not aggravate his Addison's disease.

This patient has been one of the most difficult we have had to maintain and when his data are compared with those of *patient* 4, the enormous variation in doses of extract required for the maintenance of patients with Addison's disease may be appreciated. It has been impossible to prevent periodic crises either by administration of comparatively large doses of extract (15 cc. daily) alone or combined with large doses of sodium salts. Apparently 15 cc. of the extract daily, while producing great improvement, does not give him a sufficient reserve to withstand periods of increased demand. Our experience in maintaining other patients leads us to believe that he probably

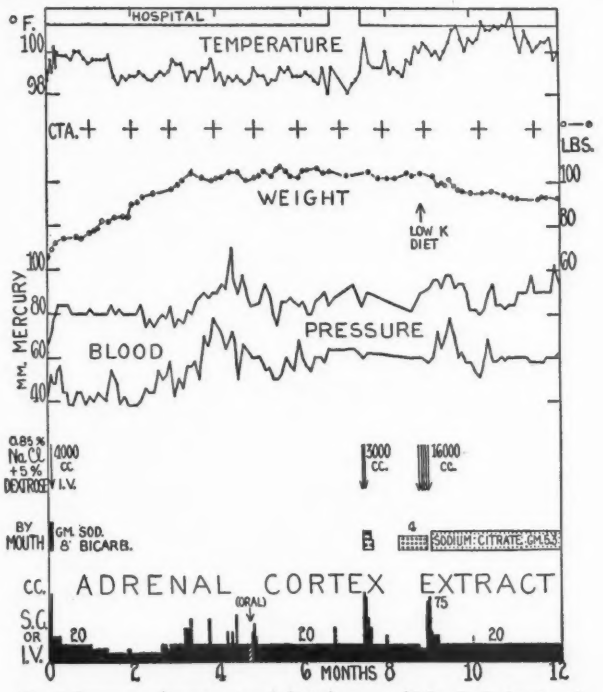

Fig. 6. Summary of treatment and clinical course of *Mrs. D. R.* (*patient* 3).

could have been kept more free from crises had it been possible to administer two or three times as much extract as we were able to secure. The necessity of administering extract is demonstrated not only by the failure of sodium salts to maintain him, but also by the difficulty sometimes experienced in reviving him from a crisis unless large doses of extract were used in conjunction with sodium salts.

Patient 3 *Mrs. D. R.* (fig. 5, 6), an American housewife, has had symptoms of Addison's disease since 1930 and has been under our observation since she entered the Cook County Hospital May 29, 1937 at the age of 29 years. At that time she was very weak and emaciated, weighing only 65 lb. She showed a generalized bronze-brown pigmentation of the skin, especially marked on the face, neck and chest. There was

a small amount of brown pigmentation on the inside of the left cheek. The systolic blood pressure was 66 mm. Hg and the diastolic, 40 mm. At the time treatment was started she was pulseless and her blood pressure could not be obtained. After being revived with large doses of adrenal cortex extract and normal salt solution administered intravenously, she was placed on a maintenance regimen of 20 cc. of extract daily and a high caloric diet. Shortly thereafter she was transferred to the Presbyterian Hospital, where she has been most of the time since. The dose of extract has usually remained at 20 cc. daily except during periods of crisis, when it has been necessary to give more. Roentgen-ray examination showed a miliary type of mottling of the lungs. Until Oct. 1937 she had an afternoon fever and tubercle bacilli were recovered from fasting gastric contents. In spite of the tuberculosis, her weight gradually rose to 107 lb. by Nov. 1937, a gain of 42 lb. The total serum base was 132.2 m.eq. per liter before treatment, and during periods of well-being, varied from 151.7 to 153.8 m.eq. per liter. Pigmentation decreased markedly. Her blood pressure rose very slowly, 80/42 being a representative reading in July 1937, 86/60 in Nov. 1937 and 90/58 in May 1938. Dec. 21, 1937, after her temperature had been about normal for 3 months, she was allowed to go home. By Jan. 12, 1938, symptoms developed again, but she failed to report until vomiting had set in. She was readmitted to the hospital Jan. 13th, where she has been ever since. It was difficult to revive her, and since readmission she has constantly shown an afternoon fever again, has had a poorer appetite and has shown a reduction in weight to about 90 lb. The basal metabolism level was −29% in Sept. 1937; −25%, Oct.–Dec. 1937; and −23% in Feb. 1938. Thyroid was not administered.

Further data on this patient are presented below in discussing the relation between the ovary and the adrenal cortex.

Patient 4, Mr. A. P. (fig. 7), a 27-year-old American railroad clerk, was referred to us Dec. 16, 1935 by Dr. Turner of the Municipal Tuberculosis Sanitarium where he had first gone Aug. 16, 1934 because of the weakness which was so marked he could not sit up for meals. His systolic blood pressure at that time was 76 mm. Hg. His skin had started to grow dark in 1931, but the change was very gradual. He vomited nearly every day for from 2 to 3 weeks after entering the sanitarium. Weakness definitely began about June 15, 1934 and vomiting some time after July 1st. In June 1934 his weight decreased from 124 lb. to 119 lb. He had had a cough for twenty years with production of a small amount of yellow sputum but no hemoptysis. Frequent examinations of the sputum and x-rays of the chest failed to show evidence of tuberculosis. There was a calcified area in the lower part of the left chest, which was thought to have been caused by empyema in 1926. At the time he reported to us he was ambulatory, weighed 133½ lb. and his blood pressure was 106/72 mm. Hg. The pigmentation of the skin had gradually decreased and no pigmentation was found in the mouth, although 4 months later it was reported that there was a small amount of brown pigment on the inner side of each cheek and on the lower gums.

No treatment was given until Sept. 1934, when a daily dose of 10 gm. of sodium chloride by mouth and 1 cc. of eschatin subcutaneously was started. The dose of sodium chloride was not changed until he left the sanitarium Aug. 28, 1935, when it was increased to 15 gm. daily. The dose of eschatin varied between 1 and 3 cc. daily, the largest dose being 5 cc. daily for a period of 10 days. For five or six weeks before leaving the sanitarium he received no cortical extract. After discharge he received 15 gm. of sodium chloride daily and 6 cc. of eschatin weekly until 2 weeks before he reported to us on Dec. 16, 1935, when the extract was discontinued. For a period of 5½ months after coming to us he received 15 gm. of sodium chloride and

3 gm. of sodium bicarbonate daily by mouth, but no extract, and on this regimen was able to return to work, although he seemed to be somewhat listless and to lack energy. In July 1936 he was given 40 cc. of Wilson's adrenal cortex extract in the course of 48 hours during what appeared to be an upper respiratory infection and was then put on 5 cc. daily in addition to the sodium salts. This has been his medication ever since except on 2 other occasions when he had a febrile reaction apparently associated with an upper respiratory infection. During these periods his dose of extract was increased slightly, but it was necessary to use only a fraction of the amount required in *F. K.* The largest amount of additional extract we have had to

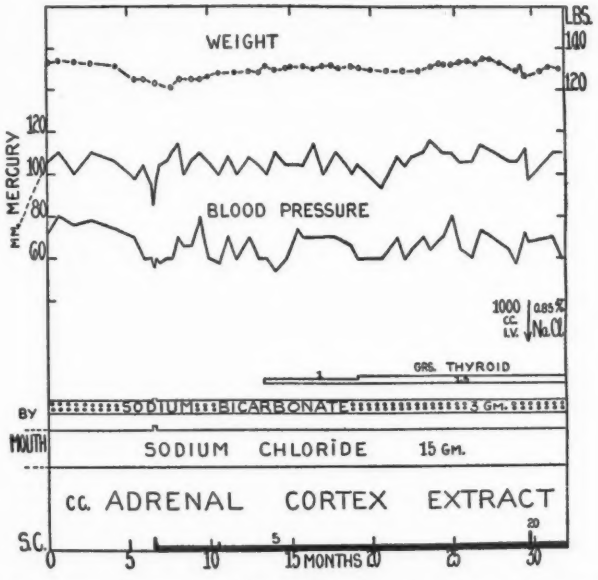

Fig. 7. Summary of treatment and clinical course of *Mr. A. P. (patient 4)*. Ht. 70½ in.

give him was 20 cc. in 24 hours. There is no doubt that the addition of extract increased his strength and made him more lively, although it did not affect his blood findings more than sodium salts alone. The concentration of total serum base was 151.6 m.eq. per liter when he first came to us and since then has varied from 151.3 to 155.9 m.eq. per liter.

The basal metabolism level during October and November 1936 was −19%. Desiccated thyroid (Wilson's) was started in a dose of 1 grain daily on Jan. 18, 1937. July 19, 1937 it was increased to 1½ grains daily and has been administered since then in this dose. His metabolism rose to a level of −7%. The effect of thyroid was definitely beneficial.

It has always been difficult to get this patient to ingest an adequate amount of food. When he was first seen by us he weighed 130½ lb. During the summer of 1936 his weight dropped to as low as 118½ lb. Since then it has gradually increased and he now weighs 130½ lb. His blood pressure varies from 100/60 to 116/80 mm. Hg. Since returning to his former position as a railroad clerk on June 2, 1936, he has continued to work.

Patients Now Dead

Three patients who have received the adrenal cortex extract for comparatively long periods have died. One patient died within 48 hours after the onset of a severe upper respiratory infection for which the treatment appears to have been inadequate; one who had active tuberculosis died suddenly, apparently from an embolus; while a third who was a physician, apparently died because he neglected his treatment. Brief case histories are given below.

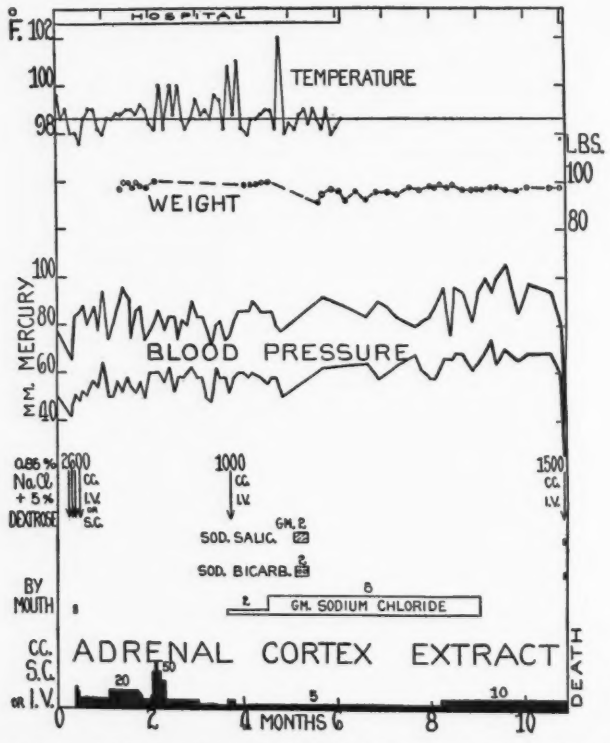

Fig. 8. Summary of treatment and clinical course of *Mrs. P. L.* (*patient 5*). Ht. 60½ in.

Patients 5, Mrs. P. L. (fig. 8), a Jewish housewife, 45 years of age, had been ill about 1½ years at the time of her admission to the Cook County Hospital Feb. 19, 1935. She had lost 40 lb. and suffered from marked weakness. She had all the characteristic findings of Addison's disease, including a blood pressure of 78/50 mm. Hg and a total base in serum of 141 m.eq. per liter. Her skin showed a generalized bronze-brown pigmentation.

After being revived with comparatively small doses of extract and normal salt solution intravenously, she was treated with doses of from 5 to 10 cc. of extract daily by the subcutaneous route, with and without additional salt by mouth. By Aug. 22, 1935 her condition had improved so much that she was able to leave the

hospital and come in every day by street car to the Central Free Dispensary for her injection. However, she was still very weak and was unable to do her housework. She remained deeply pigmented.

It was impossible to get her to eat satisfactorily and during most of the period of observation her weight varied between 90 and 100 lb. Her systolic blood pressure varied between 80 and 90 mm. Hg on nearly every determination.

After receiving 10 cc. of extract subcutaneously every day in a single dose as the only form of treatment for a period of 8 weeks, she developed an acute pharyngitis which had been present for 2 days before she reported for observation on Jan. 14, 1936. She died 6 hours after admission to the Cook County Hospital. In the light of present knowledge, her treatment was inadequate, a total of only 10 cc. of extract and of only 1000 cc. of normal salt solution being given intravenously after admission. Autopsy showed fibrocaseous tuberculosis of both adrenals with complete destruction of all cortical tissue, calcified hilum lymph glands, obliterative fibrous pleuritis, fibrous adhesions of the abdominal and pelvic organs and infectious hyperplasia and softening of the spleen.

During most of the period that she received 10 cc. of extract daily, total serum base varied from 145 to 152 m.eq. per liter. She was weak and apathetic, was unable to do her housework and was probably constantly in a mild state of adrenal insufficiency. Her poor condition is to be contrasted with that of *patient 1*, who was in good clinical condition while receiving 10 cc. of extract, although it was given in two doses instead of one. The requirement in *Mrs. P. L.* was apparently much greater than this, but it was impossible to secure more extract.

Patient 6. Mr. G. P. (fig. 9) a 49-year-old Italian laborer died suddenly apparently from an embolus after being revived from a crisis of Addison's disease associated with a marked febrile reaction with a temperature as high as 105.6° F. (by rectum). He had entered the Presbyterian Hospital on May 13, 1935 and had been referred to us by Dr. Ralph C. Brown. For 7 months before entry he had been weak, had lost 15 lb. and was troubled with pain in the right shoulder. On entry he was in a state of crisis, was very weak, unable to retain food, and had been bleeding from the rectum for 3 days. He weighed 124 lb. His blood pressure was 88/62 mm. Hg. He showed generalized brown pigmentation of the skin and the buccal mucosa was dotted with 'gray' areas of pigmentation up to 1 cm. in diameter. He was given 2000 cc. of normal salt solution intravenously on May 13th. This was the only extra salt he received in the treatment of this crisis. He received 20 cc. of Wilson's adrenal cortex extract May 13th and 14th, and thereafter the dose was gradually reduced to an average of 10 cc. daily. He improved markedly. Because of a flare-up, a dose of 4 gm. of sodium chloride daily was started by mouth on June 4th and increased to 8 gm. on Aug. 7th. A rectal ulcer was excised Aug. 27th. During his stay in the hospital he constantly showed a fever and his weight decreased to 111 lb. He was discharged from the hospital Sept. 27, 1935 feeling fairly well, although still weak. His blood pressure was 82/60 mm. Hg. From Aug. 1st (before discharge) to Oct. 29, 1935 his dose of extract was 5 cc. daily and after leaving the hospital he came in to the clinic daily for the injection. In spite of the extra salt by mouth, he became so weak that on Oct. 29th the dose of extract was increased to 10 cc. daily. For a while he felt so much better that he could chop and pile wood. However, Feb. 18, 1936 he developed an upper respiratory infection with a temperature of 101° F. He was treated with extra extract, sodium chloride intravenously and both sodium chloride and sodium bicarbonate by mouth. He recovered somewhat but remained weak and finally developed the febrile reaction mentioned above, with a

temperature of 105.6° F. (by rectum). He re-entered the hospital March 8th. With large doses of extract (60 to 70 cc. daily) and 3000 to 4000 cc. of normal salt solution intravenously per day, he improved markedly, became afebrile, and after recovering sufficiently to sit up in bed and eat his meals, died very suddenly on March 14th. Every effort was made to secure an autopsy without avail.

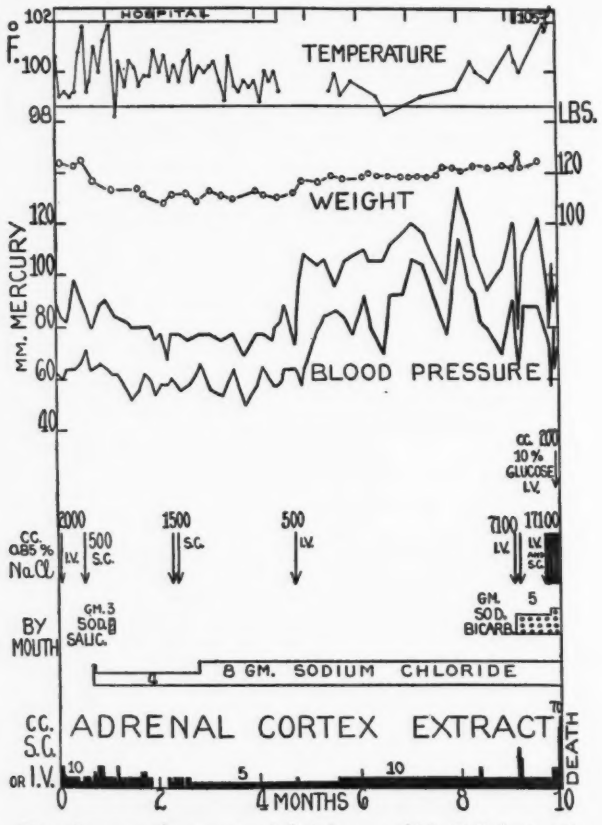

Fig. 9. Summary of treatment and clinical course of *Mr. G. P. (patient 6).*

In summarizing the data it may be seen that he had been treated for a period of 15 days with about 10 cc. of extract as the only form of therapy; and then for a period of 8 weeks, 4 gm. of sodium chloride by mouth had been given in addition. This was increased to 8 gm. daily about the time the dose of extract was reduced to 5 cc. daily, in August. Oct. 29th the dose of extract was increased to 10 cc. daily again. February 18th, 5 gm. of sodium bicarbonate daily by mouth was added to the medication. This was continued up to the time of his last relapse in March. The addition of salt and of soda by mouth did not improve his clinical condition. Throughout the

period of treatment the concentration of total serum base was on the average 149 m.eq. per liter. His appetite remained poor, he was weak and apathetic most of the time, failed to gain weight and showed a constant low grade fever with occasional flareups to 102 or 103° F., apparently the result of tuberculosis.

His condition may be compared with that of *patient* 3 who also had tuberculosis and in whom the administration of 20 cc. of extract daily was

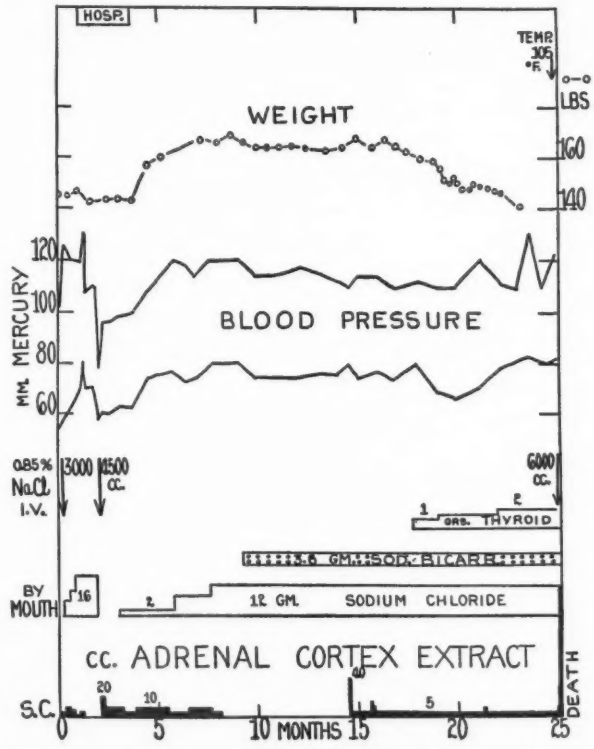

Fig. 10. Summary of treatment and clinical course of *Dr. B. K.* (*patient* 7). Ht. 74 in.

inadequate to produce an entirely satisfactory condition. It would seem that the presence of tuberculosis increases the requirement for extract.

Patient 7. *Dr. B. K.* (fig. 10), a 24-year-old American physician died partly as a result of his own negligence. Feb. 13, 1935 he entered the Presbyterian Hospital on the service of Dr. Ernest E. Irons. He was extremely weak, dyspneic and nervous. His skin showed marked generalized bronze-brown pigmentation and the buccal surfaces of his mouth showed many 'blue-gray' pigmented areas which varied from about 0.1 to 1.0 cm. in diameter. During the next few days he suffered from nausea, vomiting and hiccoughs. His blood pressure varied from 98/60 to 100/70 mm. Hg. He was given 3000 cc. of normal salt solution intravenously, 5 cc. of eschatin sub-

cutaneously every day and 4 gm. sodium chloride daily by mouth. He was discharged on Feb. 26th much improved and returned to the hospital for check-up March 23rd, when he was referred to us. He was taking 2.5 cc. eschatin daily and 13 to 16 gm. sodium chloride daily by mouth. His blood pressure had risen to 110/70 mm. Hg, his weight had not increased and he ran a slight fever of from 99 to 100° F. March 25th eschatin was omitted. He continued to feel fairly well on salt alone. April 19th salt was omitted. April 20th he was dyspneic, nauseated, nervous and weaker. At 8:30 P.M. he received 10 cc. of Wilson's adrenal cortex intravenously and this dose was repeated at 11:30 P.M. and at 7 A.M. on April 21st. It was without effect. By 8:30 A.M. on the 21st he was almost pulseless and his blood pressure had dropped to 78/58 mm. Hg. At this time normal salt solution was started intravenously and he promptly revived. He received a total of 3000 cc. during the day and it was then omitted. Extract was continued in a dose of 20 cc. daily. No salt was given by mouth. From April 26th until May 22nd he got 10 cc. of extract daily as the only form of therapy. On May 22nd, 2 gm. of sodium chloride daily by mouth was begun and on May 27th the dose of extract was reduced to 5 cc. daily. He was able to continue his work as a medical student. Aug. 3rd sodium chloride was increased to 8 gm. daily. Oct. 9th it was increased to 12 gm. daily and the dose of extract reduced to 5 cc. daily. Oct. 23, 1935 extract was omitted and 12 gm. of sodium chloride daily by mouth continued. Dec. 1st, 3.6 gm. sodium bicarbonate daily by mouth was added. He felt fairly well but could not climb stairs or exert himself without dyspnea. He remained on this regimen until May 2, 1936, when he developed nausea and dyspnea. He was revived with 40 cc. of extract daily for two days. He then felt well enough to resume the dose of 5 cc. daily. At about this time he was taking his final examinations but found it difficult to concentrate and failed in two of them. He was stronger during the summer of 1936 than during the summer of 1935, but appeared very puffy in the face and his skin and hair were very dry. Basal metabolism was found to be −35%. Thyroid, in a dose of 1 grain daily, was started Aug. 14, 1936 and increased to 1½ grains daily Sept. 16th. and to 2 grains daily on Dec. 21st. He improved remarkably, becoming more clean-cut in appearance and more alert mentally. Pigmentation changed from a grayish-bronze to a deep tan. He had no difficulty in passing his examinations and he could walk from 5 to 6 miles without undue fatigue. He began an interneship in Chicago in the autumn of 1936. He neglected his high caloric diet, did not take his extract very faithfully and lost some weight. X-ray examination and auscultation of his chest showed normal findings. He stated that he felt well. He decided to go home to Minnesota for Easter 1937. The morning after arrival his temperature was 102° F. He called his local physician, who gave him 10 cc. of extract during the day, told him to continue his sodium salts and drink water freely. That night his temperature was 103° F. and the next morning 104° F. At this time he was virtually comatose and was transferred to the hospital, where sodium chloride was started intravenously, after he had become unconscious. That night we received our first information of his illness, with a request for advice. His physician was advised to give 10 cc. of extract intravenously every hour (a supply was rushed by airplane) and to continue sodium chloride intravenously. He died the next day. (We have not yet seen a patient with Addison's disease recover after coma has set in.)

This man had had special instruction from us about Addison's disease while he was in the medical school, had read literature on the subject and was a physician. We had impressed upon him the importance of reporting to us immediately if he felt ill. In spite of this, he let matters ride until nothing would revive him.

It is our impression, as a result of repeated experiences with similar situations,

that had large doses of extract and salt solution been started intravenously at the onset of this patient's final illness, as outlined below under treatment of crises, he might be alive today. The rapidly fatal course in this crisis illustrates well that the time to begin treatment is before a crisis has advanced far. Some of his loss of weight just before his last illness may be attributed to the disappearance of his myxedematous condition as a result of the administration of thyroid, and part of it to the fact that the Addison's disease was not adequately controlled.

The autopsy was done under difficulties. Specimens of various organs were sent to us, but nothing resembling adrenal tissue was found. Tuberculosis of one tracheobronchial lymph node was found, but unfortunately no section of lung was sent. Grossly, the lungs were heavy and boggy. A large amount of bloody fluid dripped from the cut surface of the lung. No areas of pneumonic consolidation were made out grossly. The thyroid weighed 21 gm. The mesenteric lymph glands were enlarged, but the cut surface appeared normal.

Comment on deaths. In the light of recent experience, there is no doubt that the doses of extract in these 3 patients were totally inadequate. The administration of much larger maintenance doses might have improved their clinical condition sufficiently to reduce their susceptibility to infections and in 2 of them, the prompt institution of intensive treatment after the onset of a crisis, as outlined below, might have prevented the fatal outcome. We are becoming more and more impressed with the necessity of giving an adequate amount of extract.

Discussion

Symptoms and Treatment of Crises

Addison's disease, if untreated, runs a gradual downhill course, spontaneous remissions and crises alternating with one another until the patient finally dies in a crisis. It is often in a crisis that the patient first comes to the hospital and the treatment of this condition, therefore, becomes of paramount importance. A crisis is characterized by marked muscle weakness and prostration, nausea and vomiting and occasionally diarrhea. There is an accentuation of all the abnormal findings in the blood, increased excretion of sodium in the urine, marked reduction in the systolic, diastolic and pulse pressures and an increase in the intensity of pigmentation of the skin. Sometimes the symptoms are bizarre, as in the case of F. K. (*patient* 2) who, during many of his crises in the past two and one-half years, has complained of pain in the region of the lower sternum which radiates to either side of the chest, is aggravated by respiration and often accompanied by a fever with the temperature as high as 104.6° F.; there are, however, no abnormal findings in his chest on physical examination or by X-ray. The degree of nausea and vomiting varies markedly from patient to patient. Diarrhea may be a symptom with or without nausea and vomiting, which are the most common symptoms. The condition of the patient in a crisis rapidly goes from bad to worse and treatment must be started at once. It consists of the following.

a) Intravenous administration of 10 cc. or more per hour of an active adrenal cortex extract. There is no danger at present of giving too much extract.

b) Intravenous administration of about 9 gm. of sodium chloride and about 50 gm. of dextrose every 6 hours. This is given in the form of 5% dextrose in normal salt solution. Fifty cc. of 2.5% sodium citrate may be added to one liter of fluid in each 24-hour period.

c) Substitute oral administration of sodium salts for intravenous administration as soon as they can be retained. Sodium citrate is very satisfactory for oral administration.

The use of large doses of extract is very important and accomplishes more than sodium salts alone. The disappearance of nausea with treatment is striking and within 12 to 24 hours the patient can usually take food and sodium salts by mouth, and within another 2 or 3 days be placed on a maintenance program. Early in a crisis the patient may be revived by large doses of extract alone. This has been demonstrated repeatedly in *patients 1* and *2*. After vomiting has started, usually, but not invariably, it is also necessary to administer sodium salts. If the patient can be revived with extract alone, 30 to 40 cc. given in divided doses at the rate of 10 cc. per hour is usually sufficient for this purpose. This fact has been repeatedly demonstrated in *patient 2*, who has often been revived in this manner even after vomiting developed. If the patient does not revive after 40 cc. of extract has been administered in this way, sodium chloride should be started intravenously at once, but the administration of large doses of extract should be continued. If the condition of the patient is serious and nausea and vomiting marked, no attempt should be made to revive the patient with extract alone, because of depletion of the sodium reserves of the body. Extract, sodium salts and dextrose should all be started at once in the doses outlined above.

The presence of a crisis in Addison's disease is an emergency fully as great as that presented by diabetic acidosis. Nausea should always be regarded as a danger signal and in its presence appropriate treatment started at once. If treatment is begun early it is usually easy to revive the patient, but the farther the crisis has developed the less the likelihood of doing so and, if coma has set in, the patient usually dies.

Maintenance Treatment

In the treatment of a crisis the dose of extract is gradually reduced as nausea and vomiting subside and appetite returns. At the same time the oral administration of sodium salts is substituted for intravenous administration, or their administration gradually discontinued if it is planned to treat the patient with extract alone. After revival, the patient may be maintained in the following ways.

a) By administration of an active adrenal cortex extract in an adequate dose (at least 10 cc. daily and usually much more than this).

b) By prolonged administration of about 12 gm. of sodium chloride and 4 gm. of sodium citrate or bicarbonate daily by mouth. The combination works better than sodium chloride alone.

c) By a combination of *a* and *b*.

The diet should always be high in calories and, according to Wilder et al. (7, 8) and Allers and Kendall (9), low in potassium. Failure to gain weight is a serious omen and great attention should be paid to the nutritional state of the patient.

It should be emphasized that an adequate dose of the extract produces a more satisfactory clinical condition than sodium salts alone. In fact,without large doses of extract, maximum improvement can not be produced.

Oral Administration of the Extract

In two patients we have observed the effect of administering the extract by mouth. In *Mrs. D. R.* (*patient 3*) it was started before one of her characteristic premenstrual upsets (described below) was anticipated and nausea and vomiting set in within 24 hours and did not disappear in spite of increasing the dose to the equivalent of 120 cc. of extract for parenteral use daily. The extract itself seemed to produce some nausea.

In *Mrs. R. Y.* (*patient 1*) the result was more favorable. She received the extract by mouth in glycerin from April 22, 1938 up to the present time in a dose equal to 50 cc. of parenteral extract daily. Although she lost a small amount of weight at first, she continues to insist that she feels better when the extract is given by mouth in this dose than when it was given subcutaneously in a dose of 5 cc. twice a day. In interpreting this subjective improvement it should be noted that the patient was very eager to discontinue injections. She has now gone for a longer period (about 6 weeks) on extract by mouth without developing symptoms than she did after the subcutaneous administration of the extract was discontinued altogether. Her future course will be awaited with interest. The size of the dose used is purely empirical and the actual maintenance requirement can be determined only by further observation.

Low Potassium Diet

The finding of a slight increase in the concentration of potassium in the serum of patients in crisis is of interest. Wilder and his associates (7, 8) and Allers and Kendall (9) recently have reported that a high intake of potassium aggravates the condition and that diets low in potassium are beneficial. Our experience with the low potassium diet is too limited to warrant any conclusions. It concerns only two patients in the present series, both of whom have received some other form of treatment while on this diet. In the past three months *Mrs. R. D.* (*patient 3*), while on a low potassium diet has suffered from only one crisis preceding menstruation but during the same period she has also received 5.3 gm. of sodium citrate daily, so that it is difficult to say which has produced the improvement. *Mr. F. K.* (*patient 2*) has received a low potassium diet for 2 months without changing the dose of extract (15 cc. daily) and during this period has had one mild and one severe flare-up, with characteristic lower sternal and chest pain. Thus, in him, there is so far, no evidence of improvement with this diet.

The Treatment of Infections

Patients with Addison's disease appear to show an increased susceptibility to all types of infection and infections in turn increase the requirement for cortical extract just as in a diabetic patient an infection often increases the requirement for insulin. They are subject chiefly to two types of infections, upper respiratory infections and tuberculosis. When upper respiratory infections are present it is often necessary to increase temporarily the dose of extract as demonstrated by *Mrs. R. Y.*, *Mr. F. K.* and *Mr. A. P.* (*patients 1, 2* and *4*). If the condition is neglected, a crisis may develop, as in *Mrs. P. L.* (*patient 5*). The onset of an upper respiratory infection in a patient with Addison's disease always represents an emergency. The presence of tuberculosis also appears to increase the requirement for cortical extract. A comparison of *Mr. G. P.* and *Mrs. D. R.* (*patients 6* and *3*), with *Mrs. R. Y.*, *Mr. F. K.* and *Mr. A. P.* (*patients 1, 2* and *4*) illustrates that it is more difficult to produce as satisfactory a condition with doses of from 10 to 20 cc. daily when tuberculosis is present than when it is not. However, in *Mrs. D. R.* the weight did increase 42 lb. and her tuberculosis seemed to become quiescent temporarily with the administration of 20 cc. daily, but it was often necessary to give additional amounts preceding menstruation.

Basal Metabolism and Blood Pressure

In 6 of the 7 patients the basal metabolic rate was determined. Four had a low metabolism varying from -16 to -35%; and in 3 of these to whom thyroid was administered, the clinical condition definitely improved. The thyroid hormone is reported to antagonize the action of the adrenal cortical hormone in animals, but in these 3 patients no harmful effects were noted from its use. In *B. K.* (*patient 7*) the signs and symptoms of typical myxedema developed with a basal metabolism of -35%. As a result, cerebration was markedly impaired and it was only after the basal metabolism had been raised to normal with thyroid that he was able to pass the examinations in his final quarter in the medical school. His puffiness disappeared and he became more alert mentally and physically. The marked loss of weight before his death occurred later while he was serving his interneship and neglecting somewhat the treatment of his Addison's disease.

In *R. Y.*, *F. K.* and *B. K.* (*patients 1, 2* and *7*) the blood pressure rose to within normal limits during treatment, although dropping in a crisis. In *R. Y.* the systolic blood pressure did not rise beyond 105 mm. Hg until after her body weight had exceeded 100 lb. Some relation between the body weight and blood pressure was noted also in *F. K.*, *B. K.* and *D. R.* (*patients 2, 7* and *3*). In all the other patients there was a considerable amount of emaciation and the blood pressure remained low. There thus appeared to be some relationship between the general nutritional state of the patient and the level of the blood pressure. That some factor besides adrenal insufficiency is involved in maintaining the level of blood pressure in this disease is also illustrated by the fact that early in a crisis a patient may be revived without

the blood pressure showing any increase, although it may later rise to normal (e.g., *R. Y.*). Early in a crisis, nausea and vomiting are sometimes observed before any reduction in blood pressure occurs; although when a crisis is well advanced, the blood pressure is always reduced.

Relation Between Ovary and Adrenal Cortex

The data on *Mrs. D. R.* (*patient 3*) appear to furnish evidence of a relationship between the function of the ovary and that of the adrenal cortex. With the greatest regularity, while receiving 20 cc. of extract daily by the subcutaneous route, this patient developed nausea, loss of appetite and often vomiting from a few days to a week before the onset of menstruation. If these symptoms were detected early, she could usually be revived by administering additional extract (often as much as 80 cc. daily); although if they progressed very far, normal salt solution had to be administered intravenously as well. This aggravation of symptoms subsided toward the end of menstruation. Th hormonal changes occurring shortly before the onset of menstruation apparently increase the demand on the adrenal cortex. Between Feb. 26, 1938 and June 17, 1938 she had three menstrual periods without any crisis, but during this period she received, in addition to 20 cc. of extract, 5.3 gm. of sodium citrate daily and a low potassium diet.

Present Status of Treatment

The tendency for the disease to show spontaneous remissions and the length of time that sometimes elapses after omission of treatment before a crisis sets in (e.g., second omission in *R. Y.*), make it necessary to observe the effect of any form of therapy for many months before drawing conclusions about its efficacy. Periods of from one to two weeks are inadequate for this purpose, regardless of what chemical changes are observed.

It would appear that with adequate doses of extract patients may be maintained in a sufficiently satisfactory condition to carry on normal activity, provided no active tuberculosis infection is present. The smallest dose that has ever accomplished this purpose is 10 cc. daily by the subcutaneous route, and usually much more is required. The addition of sodium salts does not improve the condition of the patient if an adequate amount of extract is administered. The physical condition of the patient is better when the extract is administered in large doses than when it is not. Our patients would all probably have been in better health if we had been able to administer three or four times as much extract as we did. The failure of the pigmentation to disappear completely and the tendency of the concentration of total base in serum to remain at or just below the lower level of normal, appear to confirm this point of view and suggest that with the doses we were able to administer we had only partially controlled the deficiency.

Our experience convinces us that with sufficiently large doses of extract many patients with Addison's disease may be kept completely well. The trouble is that the source of material is so limited and thus the cost of the extract so high, that it is possible to achieve this goal in only a few patients.

It is highly desirable either that a cheaper method should be found for making the extract, or that a synthetic hormone be developed. Reports on the effect of desoxycorticosterone will be awaited with great interest.

Every patient with Addison's disease should constantly be under the observation of a physician, who has special knowledge of the disease, in order to have emergencies treated adequately as soon as they arise. Only in this way can unnecessary deaths be avoided. The greatest caution should be exercised by patients during periods of travel and at no time should they be far from a medical center where intensive treatment can be given. They should be instructed to begin sodium salts and cortical extract in large doses at the first sign of an impending crisis. Infections of all types should be avoided and every infection that does occur regarded as an emergency.

Summary

Observations with an adrenal cortex extract in the treatment of Addison's disease show that from 10 to 20 cc. daily will often maintain life for long periods without any other form of therapy, but larger amounts are probably necessary to produce optimum conditions.

In a crisis, it is important: *a*), to begin treatment at once, as delay may be fatal; *b*), to administer intravenously per hour, 10 cc. of an active adrenal cortex extract and about 170 cc. of 5% dextrose in normal salt solution, until vomiting stops and appetite returns. There is no danger at present of giving an overdose of extract.

Early in a crisis a patient may be revived by extract alone. When a crisis is well advanced, extract alone is not effective, presumably because of depletion of the sodium reserves of the body.

Following recovery from a crisis, patients may be maintained.

a), By administration of an active adrenal cortex extract in an adequate dose (at least 10 cc. daily).

b), By administration of about 12 gm. of sodium chloride and 4 gm. of sodium citrate or bicarbonate daily by mouth. (The combination works better than sodium chloride alone.)

c), By a combination of *a* and *b*.

d), The diet should be always high in calories and, according to Wilder, Allers and Kendall, low in potassium. Failure to gain weight is usually a serious omen.

An adequate dose of the extract appears to produce a more satisfactory clinical condition than sodium salts alone. If necessary, because of expense, the extract may sometimes be held in reserve for crises and patients otherwise maintained by sodium salts with or without a low potassium diet.

The dose of the extract should be increased during an infection. If no active tuberculosis infection is present, patients may be maintained in a satisfactory condition for long periods. There appears to be some relationship between the nutritional state of the patient and the level of the blood pressure. When the basal metabolism is low, raising it to normal with a suitable dose of thyroid is an important part of the treatment. Under such circum-

stances we have not observed any aggravation of the disease from its use. There appears to be a relationship between the function of the ovary and the adrenal cortex.

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Addendum

After this paper was presented, *Mrs. R. Y.* was maintained with oral administration of from 32 to 40 cc. of extract daily, equivalent in potency to from 40 to 50 cc. of extract for parenteral administration, until Dec. 10, 1938, when she developed nausea and vomiting during an upper respiratory infection. Although her weight was maintained up to the time of the infection, the concentration of total serum base had been low at least since August.

Mrs. D. R., following her menstrual period in June 1938, bled continuously up to the time of her menstrual period in July, during which she bled profusely. Her red blood cell count dropped to 2,500,000 and an Addisonian crisis set in. Following revival with extract, sodium salts and transfusions, she was started on 32 cc. of extract daily by mouth in addition to her previous regimen (20 cc. extract daily by the subcutaneous route, 5.3 gm. sodium citrate by mouth daily and a low potassium diet). Her temperature gradually dropped to normal, red blood cell count rose to 4,240,000, weight increased to 110 pounds, the blood pressure rose to 120/80, the tuberculous lesions in her lungs began to calcify and she felt better than for several years.

THE INFLUENCE OF SOME OF THE DUCTLESS GLANDS ON METABOLIC PROCESSES[1]

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THE CHEMICAL structures of the hormones of the ductless glands show them to be quite unrelated except those of the gonads and the adrenal cortex, and the symptoms of deficiency which are produced by the removal of each gland are apparently unrelated. Further inquiry into the chemical changes which are influenced, however, indicates a closely interrelated system which modifies the rate, direction and extent of the chemical processes which are involved in the metabolism of foodstuff and minerals.

Thyroxine has a high molecular weight and when attached to a protein would furnish a large, stable molecule which, through its active phenolic group, would be slightly dissociated as a negative ion. The structure is so stable and its effect is exerted for such a long time that it probably functions without destruction or alteration of the molecule. Today we do not know how thyroxine modifies chemical changes within the cells but it has been shown to increase both anabolic and catabolic processes depending on the amounts used. The metabolism of fats, carbohydrates and proteins are all affected and the distribution of minerals and water is also modified (1).

These observations suggest that the changes which are associated with a marked increase in the B.M.R. are but an exaggeration of the normal metabolic activity of the cell, without distortion and with utilization of metabolites according to their availability. The only change is in the capacity for the production of energy. However, when another hormone, as insulin or cortin, is deficient then the symptoms caused by the deficiency are aggravated and the fault in the metabolic processes becomes more marked when thyroxine is administered, even in relatively small amounts.

We may conclude that the influence of the thyroid is primarily on the rate at which cellular activity is maintained and may now inquire how the other hormones modify metabolic processes. The answer to this query again appears to be through changes in the rates of reactions but in contrast to the effect of thyroxine, the influence of other hormones is exerted on specific chemical changes and is therefore limited in extent.

The key to the interpretation of some of the influences exerted by epinephrine, insulin and cortin is provided by a study of the changes in mineral metabolism. In 1933 Loeb (2), and then Harrop and his coworkers

[1] Read before the Association for the Study of Internal Secretions, San Francisco, California, June 13, 1938.

(3), confirmed and extended earlier work that had shown that in adrenal cortical insufficiency sodium in excessive amounts was excreted but potassium was retained. The next contribution was the demonstration by Harrop and his coworkers (4) and also by investigations in the laboratory of the speaker (5, 6), that adrenalectomized dogs could be maintained indefinitely by the administration of a diet high in sodium chloride if, in addition, sodium bicarbonate or sodium citrate were given. This was soon followed by the experimental proof that normal values for potassium in the blood could be maintained in adrenalectomized dogs if the intake of potassium was restricted (7) and that the administration of even moderate amounts of potassium in the diet caused a rise of this ion in the plasma which was followed by prostration and death.

These observations have been variously regarded by several investigators. Loeb (8, 9) and Harrop (10) and their coworkers have considered the kidney to be the organ primarily influenced by cortin and that the symptoms of cortico-adrenal insufficiency were due to the loss of sodium and chloride by the kidney. Swingle (10a) and his coworkers have shown that the typical symptoms of deficiency can occur without loss of inorganic salts. They have stressed the importance of changes in blood volume and the distribution of salts and water. Britton and Silvette (10b) largely dismissed from consideration the metabolism of sodium and chloride and emphasized the changes in carbohydrate metabolism as most important. The work in my laboratory (11) has demonstrated the essential influence of sodium and chloride ions and has shown that carbohydrate metabolism can proceed in a normal manner if the supply of sodium and chloride is adequate.

Further investigations in mineral metabolism have indicated a close relation between carbohydrate metabolism and cortin on the distribution and excretion of sodium, chloride and potassium ions. We have found (12) that an intravenous injection of glucose alone will cause a marked drop in the concentration of potassium in the plasma. Regardless of the mechanism involved we have now secured evidence that it is associated with the active utilization of glucose. Normal dogs were injected for 3 hours with a 10% solution of glucose at the rate of 1 gm. per kg. per hour. At the end of the injection the potassium in the plasma was at its lowest level but within a few minutes after the high blood sugar which was present during the injection had changed to hypoglycemia the concentration of potassium began to increase until it reached a normal value.

In each case the injection produced a diuresis but without loss of glucose in the urine. The excretion of potassium varied but it was generally at a greater rate than that just preceding the injection. At no time were there any toxic symptoms or convulsions due to the low blood sugar after the injection was stopped.

When this experiment was repeated on adrenalectomized dogs very different results were obtained, which depended on 3 factors: *a*), the degree of cortico-adrenal insufficiency which depended on the length of the interval between the last administration of cortin and the time of injection of the

glucose; *b*), whether or not 0.9% NaCl was added to the solution of glucose and *c*), whether cortin was given during the injection of glucose. The influence of these 3 factors will now be given in detail (13).

Cortico-adrenal insufficiency. The influence of the degree of cortico-adrenal insufficiency on the course of events produced by the injection of glucose was most striking. If a 10% solution of glucose was injected for 3 hours into adrenalectomized dogs which had been continuously maintained in good condition after adrenalectomy either with cortin or with a diet with a high content of sodium salts, there was no untoward effect during the 3 hours of the injection. If there was an interval of 4 or 5 days after the last administration of cortin when the injection of glucose was made, anuria and acute symptoms always developed within 30 to 60 minutes after the injection was stopped. The dog became prostrated with almost complete loss of muscular activity and died within a short time unless vigorous treatment was instituted. This consisted of an injection of a 2% solution of NaCl and large amounts of cortin.

Varying degrees of cortico-adrenal insufficiency were produced by withholding cortin for intervals of from a few hours to 5 days. No adrenalectomized dog could respond in a normal manner to an injection of glucose if as many as 5 days elapsed after the last administration of cortin. At the time of the injection each dog appeared to be strong and vigorous but none could withstand the strain produced by the injection.

The influence of sodium chloride on cortico-adrenal insufficiency. When 0.9% NaCl was added to the solution of 10% glucose which was injected for 3 hours the adrenalectomized dogs responded as normal. There was a diuresis without glycosuria, the excretion of potassium was at a high rate, the increase in the concentration of potassium in the plasma after the injection was stopped was slow, and in 5 of 6 experiments the concentration of this ion 3 hours after the injection was stopped was less than that preceding the injection. No weakness or toxic symptoms developed in any of the dogs when 0.9% NaCl was used with the glucose, except in one dog which had not received cortin for 5 days previous to the injection. Anuria developed after the injection was stopped; the dog became weak and prostrated and died within 2 hours.

These results show that NaCl has a dual action on adrenalectomized animals. It maintains a high functional capacity for the excretion of potassium and water by the kidney and it affects favorably the utilization of carbohydrate by the body. These two effects are shown both by acute experiments during the 3-hour injection of glucose in 0.9% NaCl and by the experiments on the survival of dogs that were maintained after adrenalectomy, with a high intake of sodium salts but without cortin. Completely adrenalectomized dogs may be maintained indefinitely without cortin provided the intake of sodium and potassium salts is properly controlled (11). If these dogs are given a balanced diet, adequate in calories and vitamins, carbohydrate metabolism is carried on normally and the effect of a high intake of sodium ion appears to be the full equivalent of cortin.

Adrenalectomized dogs may be maintained for over 7 days without food provided sufficient amounts of sodium salts are given. During this time symptoms of cortico-adrenal deficiency do not develop and the blood sugar remains within normal limits. If sufficient NaCl is not given and food is withheld or if the dog refuses to eat there may be a decrease in blood sugar and failure to maintain carbohydrate metabolism.

It has been clearly shown that urea and potassium are easily excreted by the kidney in the presence of an adequate intake of sodium ion. One of the most striking experiments is to observe the abrupt increase in the concentration of potassium and urea in the plasma which follows the withdrawal of sodium salts from an adrenalectomized dog that has been maintained with a high intake of NaCl but without cortin (14).

Such an experiment emphasizes the important position occupied by the kidney in adrenal insufficiency but the most remarkable consequence associated with the beneficial effect of NaCl is not the high functional capacity conferred on the kidney. Of far greater significance is the fact that the fundamental chemical reactions in the tissues which involve the metabolism of fats, carbohydrates and proteins can proceed without departure from normal in the complete absence of the adrenal cortex provided the supply of sodium and chloride ions is adequate. Under some experimental conditions these inorganic ions appear to be the full equivalent of cortin but when the conditions are changed and severe stress is applied which taxes the capacity of the organism to respond then NaCl can be shown to be inadequate.

The effect of cortin on the injection of glucose. Although adrenalectomized dogs could be successfully injected with a 10% solution of glucose if 0.9% NaCl was added, still more striking results were obtained when cortin was substituted for the NaCl. The effect of cortin was to maintain the dog in a normal condition. The diuresis and excretion of potassium during and immediately after the injection was equal to or exceeded that of normal dogs depending on the amount of cortin used. With very large amounts of cortin there was glycosuria. The rise in the concentration of potassium in the plasma after the injection was stopped was slower than in the normal dog and the level to which it rose 3 hours after the injection was lower, in each case, than that immediately preceding the injection.

These results were obtained on a series of adrenalectomized dogs even 1 and 2 days following the last administration of cortin. They show that the extraordinary power exerted by cortin on the kidney permits the excretion of the potassium ion without excessive loss of sodium or chloride ions and that the excretion of sodium and chloride is probably by a different mechanism than that for the excretion of urea and potassium. In the absence of cortin the excretion of potassium and urea becomes progressively more difficult. Sodium and chloride are excreted to a greater extent by the kidney, apparently in an attempt to increase its functional capacity and a negative balance of these ions soon develops. This leads to loss of kidney function, anuria and death.

The sharply separated control over the excretion of sodium compared

with potassium has been shown by Thorn and his associates (15). The sex hormones theelin, progesterone, androsterone and testosterone all cause retention of sodium but not of potassium. The excretion of potassium, on the other hand, was not increased by these same hormones. Only with cortin-like compounds did they find the excretion of potassium accelerated.

To maintain life by the administration of NaCl requires relatively enormous amounts. This fact is in strong contrast with the physiologic activity of cortin and cortin-like compounds which, in amounts measured in thousandths of a mg. per kg. of body weight, will maintain a high functional capacity of the kidney with the excretion of potassium and urea without loss of sodium.

It has been clearly shown that in the absence of the adrenal cortex the influence of NaCl is not confined to the kidney; its effect on metabolic processes in the tissues is of equal or greater importance. The dual effect of NaCl also can be shown to apply to the influence of cortin. Its action is not confined to the kidney but is exerted through all the tissues (16).

The experimental results suggest a close parallelism between the influence of sodium and chloride ions and that of cortin but it becomes apparent at once that they modify metabolic processes through quite different channels. Changes in osmosis, ion antagonism and permeability through membranes are probably involved in the action of the sodium ion but none of these seems adequate to explain the effect of cortin.

Participation by cortin in some chemical reaction is indicated and we have many suggestive results that the chemical reaction is associated with the metabolism of potassium. The delineation of the chemical reaction must wait but it is of help to assign some chemical change which proceeds smoothly in the presence of cortin and by virtue of which cortin exerts its influence on the excretion of potassium by the kidney and the changes in the distribution of sodium and potassium in the fluids and tissues of the body. When this chemical change fails, control of potassium metabolism fails, and this in turn leads to an increase in the concentration of potassium in the plasma, which is associated with toxic symptoms, prostration and death.

The effect of potassium ion. The necessity for a rigid control over the distribution of the potassium ion in the body has been shown by the results of an injection of a 10% solution of glucose with or without NaCl or cortin into adrenalectomized dogs which were in varying degrees of cortico-adrenal insufficiency. In all of these experiments, regardless of the degree of insufficiency, the injection of glucose caused a marked decrease in the concentration of potassium in the blood plasma. This was true even if the dog was anuric.

In the experiments in which the injection of glucose into adrenalectomized dogs caused anuria there was a decrease in the concentration of potassium in the plasma, which was as great or greater than that in normal dogs, but the lowest concentration reached in the normal dogs was much less than the lowest concentration in the dogs that had anuria. Soon after the injection was stopped in the dogs with anuria there was an increase in the concentration of potassium which was both more rapid and higher than in the dogs that had a diuresis during the injection. Without exception the prostration

that occurred after the injection of glucose was stopped was related to the increase in the concentration of potassium in the plasma which was both more rapid and higher than it was in the dogs that had a diuresis during the injection. The concentration of potassium in the plasma in turn depended on the degree of cortico-adrenal insufficiency that had been produced by withholding the daily injection of cortin for varying lengths of time.

During the days when cortin was not administered there was a progressive daily rise in the concentration of urea and potassium in the plasma, and when the dogs became prostrated after the injection of glucose was stopped the level of potassium was between 6 and 9 m. eq. per l.; the lower limit of the concentration of potassium which indicated the ability of the dog to receive the injection of glucose without prostration was approximately 6.5 m. eq. per l.

The concentration of potassium in the plasma which is associated with toxic symptoms is not fixed nor is the explanation for this clear. Keith and Binger (16a) have shown that when potassium is given to normal subjects as much as 11 m. eq. per l. in the plasma may not cause toxic symptoms. In adrenalectomized dogs and in patients with Addison's disease a severe toxic effect may be associated with a concentration in the plasma of not more than 6.5 m. eq. per l. (17).

The effect of insulin and epinephrine on the potassium in the plasma. In addition to the effect of cortin and the influence of carbohydrate metabolism on the distribution and excretion of potassium, two other hormones have been found to modify the concentration of potassium in the plasma. Harrop and Benedict in 1924 (17a) demonstrated that the injection of insulin not only produced hypoglycemia but also a marked drop in the concentration of potassium in the plasma. Besides this observation, which has been confirmed by several investigators, there is good evidence that epinephrine also causes a decrease in the concentration of potassium in the plasma in man and dog (18). An immediate rise has been noted in some species of animals but we have recently shown that after prolonged injection of epinephrine in the dog the potassium is depressed to a concentration equal to that produced by insulin (12).

These results indicate some of the factors by which the concentration of potassium in the plasma may be held within narrow limits. Insulin and epinephrine decrease the concentration of potassium in the plasma and presumably force it into the cells but cortin influences the distribution in the reverse manner. The concentration within the cells is decreased and the rate of excretion is increased (19). These statements cannot be made without certain qualifications in respect to the time factor. Epinephrine affects the distribution of potassium for only a few minutes after its administration. The effect of insulin persists for a few hours and cortin may influence the distribution of potassium for 3 or 4 days after its administration. Cortin does not counteract the effect of epinephrine or insulin and its influence on the concentration of potassium in the cells may well be due to the time relationships of the factors involved.

That the same hormones which modify the concentration of potassium in the blood plasma also influence the rate of its elimination by the kidney seems probable from the work of Harrop and Benedict (17a) who found a decrease in the rate of excretion of potassium after the injection of insulin. We have confirmed this. The rigid control over the loss of potassium by the kidney has been shown in my laboratory by the administration in the drinking water of enormous doses of cortin to normal rats. This caused a prompt increase in the elimination of potassium and a markedly negative balance for a few days after which the excretion diminished until a balance with the intake was established (20).

All of the experiments which have been cited in relation to the influence of carbohydrate metabolism on the distribution of the potassium ion have been carried out without anesthesia, without the injection of potassium salts and without changes in the concentration of potassium in the plasma which were outside of physiologic limits. They form convincing evidence that a close relationship exists between the distribution and excretion of sodium and potassium and the influence of insulin, epinephrine, cortin and carbohydrate metabolism. Our insight into the mechanism of the control of mineral metabolism has recently been aided by an investigation of the influence of the pituitary and adrenal cortex on diabetes.

The 'salt and water' hormone and diabetogenic principle of the adrenal cortex. The preparation of an experimental animal by Houssay (20a), in which the symptoms of diabetes produced by pancreatectomy were ameliorated by hypophysectomy, was followed by the equally striking results of Long and Lukens (21, 22) who, by adrenalectomy, relieved the symptoms of diabetes to an extent comparable to that produced by hypophysectomy. These long-range experiments, not restricted to single substances and not clear in detail, have recently been placed on a firm foundation by the use of pure crystalline compounds which were isolated from the adrenal cortex in the laboratory of the speaker. The cortin-like compounds A and B (23) which can maintain adrenalectomized dogs in normal condition, prevent the excessive loss of sodium ion and permit the excretion of potassium ion, have been shown by Long and his associates (24) to be diabetogenic in partially depancreatized rats. The so-called salt and water hormone of the adrenal cortex and the active principle which causes formation of glycogen in the liver and glycosuria in the presence of a deficiency of insulin are identical.

This fact explains much that has been obscure. The influence of the salt and water hormone on the adrenalectomized dog has been described as dual in nature, one effect on the kidney and the other on the tissues. It is most probable that these two apparently separate effects are both dependent on the same fundamental chemical changes which occur in all tissues and organs of the body. Since these chemical reactions involve both potassium and carbohydrate metabolism, it could be anticipated that the active agent of the adrenal cortex which affects salt and water and the crystalline cortin-like compounds which affect carbohydrate metabolism might well be identical.

The proof that they are identical indicates that both NaCl and cortin

influence the same metabolic processes. What reaction is modified by cortin is a matter of speculation but Harrop and Benedict have shown that, after the administration of insulin, simultaneous with the disappearance of potassium from the blood there was also a marked decrease in the concentration of phosphate ion. We have shown that the injection of glucose alone will produce the same changes in the concentration of potassium and phosphate ions in the plasma as will insulin, except in depancreatized dogs. In 2 of 4 depancreatized dogs the injection of glucose did not decrease the concentration of either potassium or phosphate ions; the implications of this observation are clear (12). We must therefore consider the probability that after the injection of glucose or insulin, glucose and phosphate and potassium ions are all involved in some chemical reaction. Harrop and Benedict have already suggested that the decrease in the concentration of potassium in the plasma which is produced by insulin, may be due to a potassium salt which may be involved in the formation of a phosphate carbohydrate compound but whether cortin is concerned with the formation of hexose phosphates remains to be shown.

The interrelationships which have been discussed are not simple and when to the action of epinephrine, insulin and cortin the stimulating effect of thyroxine is added, and finally, the modifications of the activity of the glands exerted by the family of hormones in the anterior lobe of the pituitary body, the result is one of magnificent proportions. The endocrinologist can contemplate the scope of his special field of endeavor with assurance that he will have important problems to solve for an indefinite period, and the biochemist need not fear the fast approach of the time when his work will be finished.

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THE STIMULATING ACTION ON METABOLISM AND HEART BEAT OF VARIOUS THYROID PREPARATIONS, DETERMINED IN THE THYROIDECTOMIZED RAT

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THYROID PREPARATIONS are generally classified among the dangerous drugs because of their potential toxicity, which means that at a certain dosage they are likely to produce unpleasant and even alarming symptoms. Such manifestations are the same as are observed in hyperthyroidism, namely, restlessness, nervousness, excessive sweating, palpitations and increased heart rate. Whether or not such action is definitely connected with the increase in metabolism has never been decided. If this should be the case it would speak in favor of the theory that assumes a single active principle in the gland, the action of which leads to a variety of manifestations. Contrariwise, if it were possible by the use of fractionated material to produce one or several of the secondary symptoms independently from metabolic increase, it would speak strongly for the existence of two different substances—at least in the material employed, if not in the gland itself. Of the side-effects mentioned, only the heart beat lends itself to a quantitative study.

The heart stimulation in thyroidectomized rats by thyroid material and thyroxine has been investigated by Fishburne and Cunningham (1). Since their work did not include metabolic tests, their results do not convey any knowledge with respect to our problem.

In a previous publication (2) we reported a comparative study of the action of various thyroid preparations on the metabolism of the thyroidectomized rat. It was shown, that calculated on thyroxine content, thyroid globulin is of a higher efficacy than dried thyroid substance, the latter again being more active than pure thyroxine (Squibb), and that the acid-soluble and insoluble hydrolysates of the globulin fraction have an intermediate position. (We refer to this article in order to avoid lengthy repetitions of reports.)

It is regrettable that records of the heart beats have not been kept from the very beginning of this work. However, they are available from the larger part of the investigation and they have been used in this paper together with some additional work.

The normal rat, about 80 days of age, has a heart rate of 300 to 330 beats per minute. The oxygen consumption at that time is from 1400 to 1600 mg. O_2 per hour per kg. weight. Four weeks after the operation the heart beat is reduced to 180 to 200 and the metabolism to 900 to 1200.

The object of the study was to observe to what extent heart stimulation and metabolic increase show proportionality in the use of various thyroid preparations.

An increase of metabolism to values between 1400 and 1600, corresponding to 45–50% increase, would return the thyroidectomized rat to the level of the normal animal. The heart rate in this case should be raised to 300 to 330, if a proportionate action should exist.

In counting the heart beat, we found the mechanical devices available to us to be unsatisfactory. It is of primary importance that the animal is comfortable and restful. This was obtained by holding the rat snugly between the bent arm and chest. The animal usually places the head into the corner at the elbow and takes a sleeping position. The feeling of the heart beat by placing the finger tip close to the sternum on the left side requires some skill, especially in the untreated thyroidectomized rat, where the beat is rather weak. The determination was done by counting 60 beats[1] and registering the time with a stop watch. Each investigator performed several countings and was checked by the independent determinations of the coworkers. The results agreed within 10 points per minute, with exception of values above 360. At that rate accurate counting becomes difficult and wherever we recorded such figures we consider them simply as 'higher than the heart beat of the normal rat.'

The following tables show the relation between metabolic increase in response to a given medication (the dose being calculated per 10 gm. weight and given for 3 consecutive days) and the heart beat before and after treatment. While routinely the rats were used only a single time for a test, 3 rats have been carried through for a more extended period.

TABLE 1

No.	Dose γ/10 gm./on 3 days	Thyroxine O_2 consumption % increase	H. B. before	H. B. after
1	3	45	188	225
2	3	48	180	240
3	3	47	182	257
4	3	40	189	257
5	3	45	180	248

Tables 7, 8 and 9 show the history of these individual rats, which have been treated alternatingly with the various substances. These tables show also the time found necessary until the effect of treatment has disappeared.

DISCUSSION

The results show quite definitely that the ratio between acceleration of heart beat and increment in metabolism varies fundamentally between the various products, although stimulation of metabolism is always connected

[1] This is done by counting 6 times to 10, as the composite words of figures do not permit fast counting.

with a certain degree of heart action. Thyroxine showed a relatively slight effect on the heart, as the count was maximally only half way up to normal, when the metabolism reached values corresponding to the normal rat. Thyroid globulin behaved in a perfectly analogous way. Occasionally a definitely increased metabolism was observed concomitantly with quite insignificant heart effect.

TABLE 2

No.	Dose γ/10 gm./on 3 days	Thyroid globulin		H. B. before	H. B. after
		Equivalent thyroxine	O_2 consumption % increase		
1	40	0.1	10	180	211
2	40	0.1	10	189	232
3	40	0.1	4	180	220
4	40	0.1	10	189	220
5	40	0.1	3	184	215
6	40	0.1	7	180	189
7	50	0.125	17	180	218
8	50	0.125	13	185	240
9	50	0.125	20	180	232
10	50	0.125	15	180	225
11	50	0.125	19	180	200
12	50	0.125	18	180	200
13	50	0.125	16	180	225
14	50	0.125	21	185	200
15	50	0.125	20	190	212
16	50	0.125	14	185	225
17	75	0.19	20	180	190
18	75	0.19	26	180	212
19	200	0.5	43	191	225
20	300	0.75	48	185	248
21	300	0.75	46	180	240
22	300	0.75	50	194	248
23	300	0.75	44	189	248
24	300	0.75	43	175	257

TABLE 3

No.	Dose γ/10 gm./on 3 days	Acid-soluble fraction		H. B. before	H. B. after
		Content of thyroxine	O_2 consumption % increase		
1	1000	0.15	0	186	232
2	1000	0.15	5	187	248
3	2000	0.30	14	170	288
4	2000	0.30	15	189	240
5	2000	0.30	9	182	257
6	2000	0.30	10	185	248
7	2000	0.30	13	180	257
8	3000	0.45	30	182	300
9	3000	0.45	20	180	289
10	3000	0.45	34	180	300
11	3000	0.45	28	186	230
12	3000	0.45	26	182	290
13	6000	0.9	37	184	330
14	6000	0.9	35	186	327
15	6000	0.9	26	186	315
16	6000	0.9	32	189	330
17	6000	0.9	27	179	313
18	10000	1.5	36	200	327

TABLE 4

No.	Dose γ/10 gm./on 3 days	Acid-insoluble (thyroxine) fraction		H. B. before	H. B. after
		Content of thyroxine	O_2 consumption % increase		
1	7.5	0.25	5	180	277
2	22.5	0.75	0	185	300
3	22.5	0.75	16	184	277
4	30.0	1.0	20	180	327
5	30.0	1.0	13	184	342
6	30.0	1.0	20	180	342
7	30.0	1.0	21	196	360
8	37.5	1.25	29	182	327
9	37.5	1.25	34	180	344
10	37.5	1.25	26	186	342
11	37.5	1.25	21	182	355
12	37.5	1.25	23	180	360
13	40.0	1.33	20	190	342
14	40.0	1.33	14	185	340
15	40.0	1.33	19	190	380
16	40.0	1.33	13	184	378
17	40.0	1.33	18	200	315
18	60.0	2.00	17	190	370
19	60.0	2.00	23	180	360
20	60.0	2.00	27.5	190	342
21	80.0	2.66	31	184	300
22	80.0	2.66	8	190	360
23	90.0	3.00	28	186	340
24	150.0	5.00	30	182	400
25	150.0	5.00	36	180	360
26	150.0	5.00	20	186	360

TABLE 5

No.	Dose γ/10 gm./on 3 days	Commercial Thyroid Powder U.S.P. XI		H. B. before	H. B. after
		Content of thyroxine	O_2 consumption % increase		
1	320	0.3	26	182	342
2	320	0.3	26	180	360
3	320	0.3	30	189	327
4	320	0.3	35	183	334
5	320	0.3	35	182	360
6	1280	1.2	53	182	360
7	1280	1.2	40	191	345
8	1280	1.2	50	189	345
9	1280	1.2	42.5	187	377

Both hydrolysates of the globulin, on the other hand, have a decided preference toward heart action; a heart rate increase by 50 points, or more, was occasionally coexistent with unchanged metabolism. At metabolic levels still far below normal, the beat reached and exceeded the normal figures. This was especially conspicuous with the acid insoluble fraction.

Dried whole thyroid preparations uniformly showed that the action on the heart exceeded that on metabolism. Comparing the thyroid globulin with the total gland powder, it seems to be evident that the gland contains a

TABLE 6

No.	Dose γ/10 gm./on 3 days	Thyroid with 4.65% I		H. B. before	H. B. after
		Content of thyroxine	O_2 consumption % increase		
1	133	0.25	29	187	400
2	133	0.25	31	192	378
3	133	0.25	33	190	360
4	133	0.25	33	194	360
5	133	0.25	25	191	360
6	167	0.32	36	183	355
7	167	0.32	44	182	350
8	167	0.32	44	188	363
9	400	0.75	52	189	420
10	400	0.75	40	185	380
11	400	0.75	52	189	400
12	668	1.28	58	189	400

TABLE 7. RAT NO. 15693

Date	Weight	Heart beat	B. M. O_2 γ/hr./kg.	B. M. % inc.	Treatment
3/8	170	310			Thyroidectomy
4/19	160	180	920		
4/22	158	180	929		
4/25–26–27					50 γ thyroid globulin per 10 gm. per day, equal to 0.125 γ thyroxine
4/29	164	200	1100	18	
5/11	175	215	1071		
5/19	171	189	1045		
6/3	151	180	900		
6/6–7–8					3 γ thyroxine per 10 gm. per day
6/10	153	240	1355	48	
6/14		276			
6/16	178	240	1265		
6/24	197	189			
6/29	187	179	1015		
7/6	185	189	900		
7/7–8–9					6000 γ acid-soluble fraction=0.9 γ thyroxine
7/11	188	330	1160	32	
7/28	232	327			
8/22	214	257			
9/2		180			
9/6	199	181	910		
9/8–9–10					60 γ acid-insoluble fraction=2 γ thyroxine
9/12	200	378	1080	20	
10/12	215	180	920		
10/13–14–15					320 γ thyroid U.S.P.=0.3 γ thyroxine
10/17	207	350	1170	27	
10/26	229	300	1010		
11/2	233	300	983		
11/9	238	200	955		
11/16	235	194	900		
11/17–18–19					300 γ thyroid globulin per 10 gm. per day; equal to 0.75 γ thyroxine
11/21	224	232	1310	45	

TABLE 8. RAT NO. 16003

Date	Weight	Heart beat	B. M. O_2 γ/hr./kg.	B. M. % inc.	Treatment
5/25	225	327			Thyroidectomy
6/21	210	180	900		
6/23	208	184	900		
6/23–24–25					3 mg. acid-soluble fraction=0.45 γ thyroxine
6/27	209	300	1170	30	
6/28	214	300			
6/29	219	300			
7/20	226	276	1125		
7/28		300			
8/9	230	276	974		
8/22	238	276			
9/15	231	200	1075		
9/21	235	200	1000		
9/28	237	210	930		
9/29–30 & 10/1					40 γ acid-insoluble fraction=1.33 γ thyroxine
10/3	238	315	1095	18	
10/14	259	313			
10/19	247	300	1000		
10/26	257	300	995		
11/1	259	278	926		
11/9	255	205	975		
11/15	264	200	950		
11/30	240	194	900		
12/1–2–3					2.5 γ thyroxine per 10 gm. per day
12/5	236	280	1350	50	

TABLE 9. RAT NO. 15960

Date	Weight	Heart beat	B. M. O_2 γ/hr./kg.	B. M. % inc.	Treatment
5/16	210	327			Thyroidectomy
6/7	194	189	910		
6/9	192	189	930		
6/9–10–11					3 γ thyroxine per 10 gm. daily
6/13	193	257	1350	45	
6/14		266			
6/17	201	240	1315		
6/24	240	250			
6/29	214	248	1048		
7/13	211	200	900		
7/14–15–16					10000 γ acid-soluble fraction=1.5 γ thyroxine
7/18	217	327	1220	36	
7/28	261	300			
8/22	233	257			
9/14	209	194	900		
9/15–16–17					133 γ thyroid of 0.465% I=0.24 γ thyroxine
9/17	206	360	1200	33	
10/14	243	327			
10/19	227	200	960		
10/26	237	200	960		
10/26–27–28					300 γ thyroid globulin=0.75 γ thyroxine
10/31	230	232	1385	44	
11/9	237	200	1130		
11/16	238	194	1010		
11/30	240	190	900		

second hormone, a substance of specific action on the heart, that has been eliminated in the process of extraction and purification of the globulin, perhaps leaving it slightly contaminated, just sufficient to produce a moderate heart effect. This assumption of contamination with a second hormone, however, becomes dubious in view of the fact that the crystallized thyroxine (Squibb), claiming to be a chemically pure substance, shows an exactly analogous heart action. The observation that the hydrolysates, prepared from the thyroid globulin, have quite a conspicuous effect on the heart, makes the problem more involved. Since hydrolysis causes the formation of split products of increased activity on the heart, the possibility exists that spontaneous hydrolysis might occur in the gland after death, leading to the formation of cardio-stimulating substances. The question thus remains, whether the heart stimulator in the gland powder is a by-product or an original hormone.

The presence of a second substance in these preparations might explain the rather erratic results in metabolism tests obtained occasionally with the acid-insoluble fraction. An unwontedly low response in metabolic increment was associated with an equal stimulation of the heart as obtained in those tests which showed a good metabolic increase. It can be visualized that the substance acting on the heart was physiologically active in other respects as well, for example, had influence on the intestine. This heart stimulator was obviously absorbed in every instance, since that effect was never missing, but if one presumes that its action occasionally interfered with the absorption of the other principle, the varying results are easily understood. This hypothesis gives at least some explanation as to why of all products on test this was the only one that could not be assayed by metabolism measurements.

A variable influence of thyroid preparations on the intestine has been claimed by Buzzo, Agostini de Muñoz and Bayley Bustamante (3). Their assertion that thyroxine does not show such effect is in agreement with our hypothesis, but the statement that thyroid globulin does influence the intestinal muscle contradicts it. There is of course the question whether their globulin was as highly purified as our preparation, and their experiments did not deal with the question of absorption. Since most thyroid products, used clinically, are not of a very definite uniformity, it is not surprising that clinicians ascribed different degrees of 'toxicity' to the various products used.

Thyroxine has been blamed for a strong effect on the heart. Our experiments seem to disprove this. But the discrepancy is easily explained by the fact that commercial thyroid tablets do not contain the crystallized product, but a crude compound that probably closely resembles our acid-insoluble fraction.

Our observations are decidedly at variance with the widespread opinion which is expressed by Harrington (4), as follows: "The effect of thyroid secretion may be studied in a variety of aspects, all of which, however, are referable to its general property as a controller of metabolism."

The continued experiments on individual rats showed that the tests could be repeated on the same animals with identical results as obtained when a single test was performed, provided a sufficient time for depletion was

allowed. It appears that 4 weeks was the required minimum, but that in some instances considerably more time was necessary before both heart beat and metabolism returned to the pre-treatment minimum. Whether there is a difference in elimination between the various preparations used and to what extent the two observed functions coincide in their rate of regression cannot be decided from the few data presented.

An observation, that is not directly related to the presented study may be mentioned as being of interest. The thyroidectomized rats did not attain the weight of normal rats of their age. After each treatment, disregarding the kind of thyroid preparation, there was an increase of weight. As the effect of the treatment wore off, a tendency towards losing weight again was observed. This is in agreement with Janney's (5) observation of an anabolic effect of the thyroid, and also with Maillet's (6) recommendation of thyroid treatment of hypotrophic infants.

SUMMARY

Treatment of thyroidectomized rats with various thyroid preparations does not act on heart beat and metabolism in an analogous manner. Doses of thyroxine or thyroid globulin that will elevate the metabolism to levels of the normal animal stimulate the heart rate only half way to the corresponding level. With dried whole thyroid the acceleration of the heart rate precedes somewhat the metabolic increment and reaches hyperthyroid-like tachycardia, when the latter arrives at the normal-rat value. Hydrolysis split products of the globulin cause far more pronounced heart stimulation, leading to normal and higher beat figures, while the metabolism lags behind. Considerable stimulation of the heart was occasionally observed with these latter preparations, when the metabolism seemed not to be influenced at all.

Although it is evident that split products obtained by hydrolysis from the relatively weak cardio-stimulating globulin have a strong heart action, it cannot be said whether the same substances are also present in the original gland, and if so, whether one is dealing with a genuine hormone, or with some secondary product perhaps formed by postmortem changes.

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INTERACTIONS OF ESTRONE AND PROLACTIN WITH SPECIAL REFERENCE TO THE EFFECT OF ESTRONE ON THE PIGEON CROP-GLAND RESPONSE

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RIDDLE AND BRAUCHER (1) were the first to show that injections of anterior pituitary extracts would cause enlargement and secretion of the crop-glands of the pigeon. It had been known since the pioneer experiments of Stricker and Grueter (2) that lactation could be initiated, in animals possessing mammary glands capable of function, by injections of extracts of anterior lobe, and experiments (3, 4, 5) indicated that the same anterior lobe hormone (called 'prolactin' by Riddle) was capable of causing initiation of lactation and growth of the pigeon crop-glands.

It may be pointed out that, on the one hand, there is at present no absolutely conclusive evidence of the identity of the crop-stimulating and lactogenic factors of the anterior pituitary, while on the other hand there is a certain amount of evidence to the contrary. Thus Nelson (6) reported widely different ratios between the potencies in pigeon units and rabbit units (the latter based on the initiation of lactation in the pseudo-pregnant rabbit) of various anterior lobe extracts. Further, Folley and Young (7) have studied, on lactating cows, the lactogenic effects of AP extracts prepared in different ways and have found that while the correlation between pigeon crop-stimulating activity and lactogenic activity was poor, a much better correlation existed between the latter and the glycotropic activity (8). In view of this, they suggested that at the present stage it would be preferable to use the term 'prolactin' in respect of crop-stimulating activity only.

It is well established that administration of estrogenic hormones to lactating animals results in complete or almost complete inhibition of lactation (for citations see 9) which may be partly due to suppression, by the action of the circulating estrogen, of the secretion of lactogenic hormone by the anterior lobe of the pituitary gland, and partly to a direct action on the mammary gland (6). In view of this, and since Nelson (10) was able to inhibit the lactogenic action of anterior lobe extracts in guinea pigs by simultaneous injections of estrogens, Folley and White (11) were led to examine the effect of estrogen administration on the crop-gland response, with the result that the response was found to be considerably less in birds treated simultaneously with prolactin and estradiol monobenzoate than in controls given prolactin but no estrogen.

Before this finding can be taken as providing more evidence in favor of the identity of the crop-stimulating and lactogenic hormones of the anterior lobe, further information about the mechanism of this effect is desirable. The work described below was undertaken with two main objects in view; first, to attempt to elucidate the mechanism of the inhibiting effect of estrogens on the crop-gland response, and second to study the effect quantitatively in terms of estrone, in order to provide a standard with which to compare the potencies of other estrogens. These experiments further gave an opportunity of following up the preliminary observations of Folley and White (11) on the antagonism between estrogens and prolactin in their effects on the body weight of the pigeon. For the sake of completeness, observations on the effect of prolactin on the vaginal response to estrone in the ovariectomized mouse were also made.

Technic used for studying the effect of estrone on the crop-gland response. The method of study adopted consisted in principle of measuring the effect of different doses of estrone on the response of the pigeon crop-gland to a constant dose of prolactin. The criterion of crop-gland response used was the increase in the weight of the crop-glands after administration of prolactin to pigeons under the conditions recommended by Rowlands (12) for the assay of prolactin. The pigeons were of 'homer' type but of mixed age and breed, groups of 20 being used for all experiments except one in which the two groups (see below) were limited to 16 to economize estrone. In a few groups one or two birds died during the experiment. Each bird received 6 daily injections of 1 cc. of aqueous solution containing a suitable dose of prolactin. The injections were subcutaneous and always given in the axillary region on the right side. Twenty-four hours after the last injection the birds were killed with coal gas, weighed and sexed. The crop glands were dissected out, care being taken to take only the two thickened areas and to free these of adherent fat. They were fixed for 24 hours in Bouin's fluid and then dehydrated for 3 hours in 70% alcohol, from which they were weighed after pressing in soft muslin.

Immediately following each prolactin injection, every bird belonging to an experimental group was given an injection of estrone dissolved in sesame oil, the injections being subcutaneous and given in the axillary region on the left side. In all experiments a control experiment was performed at the same time, each bird in the control group receiving similar injections of prolactin followed by injections of the appropriate volume of sesame oil. All experiments except 3 were done singly, a control experiment being run for each. On one occasion, it was possible to run the remaining 3 experiments together, one control group serving for all 3. For each experiment, from the known mean weight of the crop glands of pigeons which had received 6 daily injections of 1 cc. sesame oil only (this was determined on a group of 20 birds in a separate experiment), it was possible to calculate *a*) the absolute crop increase prevented by the estrone and *b*) this quantity expressed as a percentage of the mean crop weight increase in the controls. The latter quantity is hereinafter called the 'percentage inhibition' and it may be regarded as a measure of the percentage of prolactin inhibited. By running control groups parallel with each experimental group, variability of response among different batches of birds was to some extent corrected for.

Riddle et al. (3) and Rowlands (12) have shown that with a given dosage of prolactin and method of administration the weights of the crop glands obtained at

autopsy from a number of treated pigeons are positively correlated with their body weights. Since it was impossible to obtain adequate supplies of birds of uniform body weight, it was considered advisable to express the weight of the crop gland of each bird as a percentage of its body weight at killing. As a further precaution each experimental and its corresponding control group were matched as regards the body weights of the birds comprising them. This was done by weighing about 50 birds before the experiment and selecting the 40 most uniform, from which the pigeons were chosen in pairs in order of weight, beginning with the 2 heaviest, one being placed in each group. Supplies of pigeons of known age could not be obtained, but it was considered that by using fairly large groups chosen as just described, birds of different ages would be fairly evenly distributed as between any experimental group and its control group.

The prolactin preparation used throughout this work was prepared from acetone dried anterior lobe powder (B.D.H. Ltd.) by the modification of the method of Bates, Riddle and Lahr (13) described by Young (14).[1] There were two main series of experiments. Each bird in series 1 received 1.5 mg. of the preparation daily and in series 2, 3.0 mg. These doses were chosen as giving values for the mean crop-gland weight per 100 gm. body weight falling on the rising portion of the dose response curve shown in figure 1. This curve was obtained from experiments on groups of 20 or more birds which received 6 daily doses of 1 cc. sesame oil as well as appropriate doses of prolactin, the daily dose of which was always dissolved in 1 cc. of aqueous solution.

Series 2 was divided into two sub-series, (a) and (b) in which the daily doses of estrone were dissolved in 0.5 cc. sesame oil and 1 cc. sesame oil respectively. Table 1 gives a summary of the treatment accorded each group.

TABLE 1. INHIBITION BY ESTRONE OF THE RESPONSE OF THE PIGEON CROP GLAND TO PROLACTIN

Daily dose of estrone mg.	No. of birds in exper. group	No. of birds in control group	Mean crop wt. (W_E) gm./100 gm. bird (exper.)	Mean crop wt. (W_c) gm./100 gm. bird (control)	Per cent inhibition $\frac{100(W_c-W_E)}{W_c-W_u}$*
Series 1. 1.5 mg. prolactin daily. Daily oil volume 1.0 cc.					
0.20	20	20	0.739	0.929	32.3
0.50	20	18	0.627	0.957	53.6
1.00	17	18	0.620	0.957	54.7
2.00	18	18	0.627	0.957	53.6
Series 2a. 3.0 prolactin daily. Daily oil volume 0.5 cc.					
0.10	20	20	1.338	1.401	5.9
0.50	20	19	1.137	1.420	26.2
1.00	16	16	0.920	1.579	53.2
Series 2b. 3.0 mg. prolactin daily. Daily oil volume 1.0 cc.					
0.75	20	20	1.101	1.427	30.0
1.50	20	20	0.803	1.355	54.4
2.00	20	19	0.840	1.361	51.1

* Mean weight (W_u) of crop glands from 20 birds each given 6 daily subcutaneous injections of 1 cc. sesame oil=0.341 gm./100 gm. bird.

RESULTS AND DISCUSSION

Inhibition of the crop-gland response by estrone. The results are summarized in table 1 and expressed in terms of the amount of crop growth prevented, in figure 2. It will be seen that with a given daily dose of prolactin, the amount of crop growth prevented increases to a maximum as the daily dose of es-

[1] For the gift of this material I am greatly indebted to Dr. F. G. Young.

trone rises, and that a lower daily dose of estrone sufficed to produce the maximum inhibition of the crop growth resulting from daily doses of 1.5 mg. prolactin than was necessary to produce maximum inhibition when the daily dose of prolactin was 3.0 mg. As regards the effect of oil volume on the

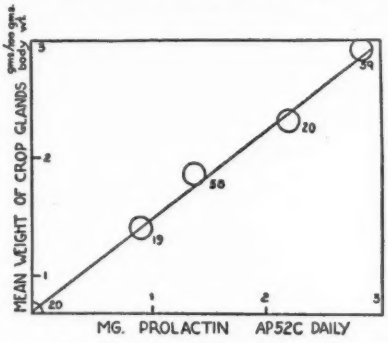

Fig. 1. Dose—response curve for prolactin preparation AP52C. The numbers against the points indicate the number of birds in each group.

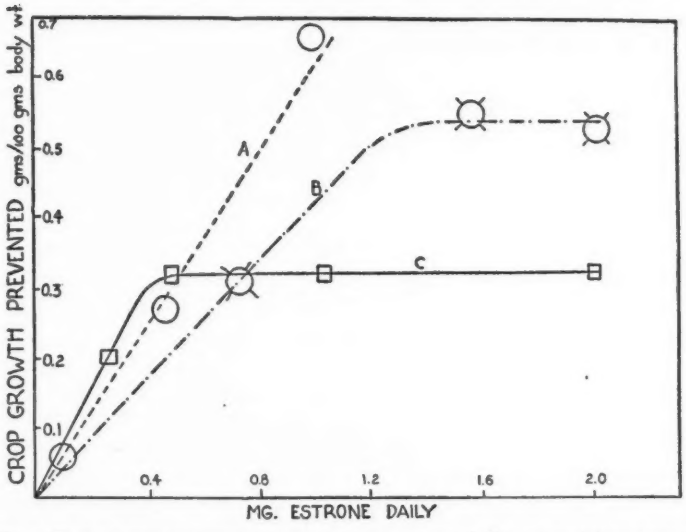

Fig. 2. Crop growth prevented by estrone administration. A. Pigeons receiving 3.0 mg. AP52C daily. Daily dose of estrone was dissolved in 0.5 cc. sesame oil. B. Pigeons receiving 3.0 mg. AP52C daily. Daily dose of estrone was dissolved in 1.0 cc. sesame oil. C. Pigeons receiving 1.5 mg. AP52C daily. Daily dose of estrone was dissolved in 1.0 cc. sesame oil.

inhibitory action of estrone, the experiments with 3 mg. prolactin daily show that the amount of crop growth prevented by any daily dose of estrone up to 1.0 mg. dissolved in 0.5 cc. sesame oil is higher than if the oil volume is 1.0 cc. daily. The rate of absorption of estrone from the site of injection is evidently, therefore, an important factor. It was impossible to trace the curve

corresponding to series *2a* beyond the point shown in figure 2 owing to the impossibility of obtaining more concentrated solutions of estrone.

Inspection of figure 2 shows that the maximum amount of crop growth prevented by estrone was considerably higher when the daily dose of prolactin was 3.0 mg. than when it was only 1.5 mg. When the curves showing the results expressed in terms of percentage inhibition are examined (fig. 3), a further interesting fact is immediately apparent, namely, that the maximum percentage inhibition obtained in any of these experiments was constant and amounted to approximately 54%. It is interesting to note that the per-

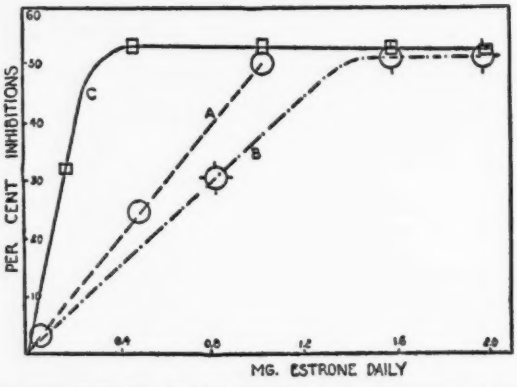

Fig. 3. Percentage inhibition of crop growth caused by administration of estrone. A Pigeons receiving 3.0 mg. AP52C daily. Daily dose of estrone was dissolved in 0.5 cc. sesame oil. B. Pigeons receiving 3.0 mg. AP52C daily. Daily dose of estrone was dissolved in 1.0 cc. sesame oil. C. Pigeons receiving 1.5 mg. AP52C daily. Daily dose of estrone was dissolved in 1.0 cc. sesame oil.

centage inhibition was about 54% in the experiment in which 3.0 mg. prolactin and 1.0 mg. estrone in 0.5 cc. sesame oil were administered daily, despite the fact that in this experiment the response of the control group to 3.0 mg. prolactin daily was somewhat higher than in the rest of the experiments in this series (series *2a*).

It therefore appears that under the conditions of these experiments the maximum inhibition of the pigeon crop-gland response to the prolactin preparation, which can be brought about by administration of estrone, is constant at about 54%, even with a relatively low dose of prolactin. This is in contrast to the findings of Young (15) in a study of the inhibition of the crop-gland response by an anti-prolactin serum. From his statements it may be inferred that by use of such sera it is possible to cause complete inhibition of the crop-gland response. The mechanisms of the two types of inhibition are, however, undoubtedly different.

Folley and White (11) observed a statistically significant difference between males and females as regards the percentage inhibition of the crop response caused by estradiol monobenzoate. This, however, was largely due to a significant difference of the mean responses of male and female birds to

prolactin plus sesame oil. This result must have been due to some chance factor, since in the present more extensive study, no indication of any sex difference as regards the percentage inhibition has been found.

The data of figure 3 enable the definition of suitable conditions for comparing the power of other substances to inhibit the response of the pigeon crop gland with that of estrone. Under the conditions of these experiments the method most economical in material is to determine the daily dose of the unknown, administered in 1.0 cc. sesame oil, necessary to cause 30% inhibition of the response of the crop gland to 6 daily doses of 1.5 mg. of the prolactin preparation in 1 cc. aqueous solution.

Though at the present stage it is impossible to give a complete interpretation of the results in figures 2 and 3, it is of interest to indicate briefly some of the possible theories which might be put forward to account for various aspects of them.

a) Estrogens inhibit the crop response to prolactin, but at the same time cause development of some crop tissue. This possibility was shown to be invalid by an experiment in which 10 pigeons were each given 6 daily injections of 1 mg. estrone in 1 cc. sesame oil. The mean crop weight/100 gm. body weight at the end of the injections was equal to that for untreated birds in this laboratory, namely 0.34 gm.

b) Prolactin exerts two distinct effects on the pigeon crop gland, both leading to an increase in weight, but one only of which is inhibited by estrone. Since the maximum inhibition was found to be the same at two levels of prolactin dosage, if this theory were true the proportions of the total response contributed by both components would appear to be the same at all levels of prolactin.

c) The inhibition is due to 'suppression' of the pituitary functions by the injected estrogen, leading to a condition partially resembling that following hypophysectomy. Estrogen treatment is well known to lead to such a condition in the rat (16) and the observations of Schooley, Riddle and Bates (17) who found that hypophysectomised pigeons are much less sensitive to prolactin than intact ones, would support this theory. However, it is a little difficult to see why the maximum inhibition should never be more than about 50%, even with high doses of estrone and relatively small doses of prolactin, since Schooley et al (17) found that hypophysectomized birds gave only about one-eighth of the response of intact ones, while Gomez and Turner (18) observed no response at all to prolactin in completely hypophysectomized birds. On the other hand, the fact that 100% inhibition was never observed in these experiments might be due to slower absorption of estrone from the site of injection, than of prolactin.

d) The prolactin preparation used contains more than one crop-stimulating hormone, the action of one only of which is inhibited by estrone. This theory offers a good explanation why the maximum percentage of crop-stimulating activity inhibited in these experiments was the same at both levels of prolactin dosage.

The effect of prolactin on the vaginal response of the ovariectomized mouse to estrone. In view of the fact that estrone has been shown to inhibit at least one response to prolactin it was of obvious interest to determine whether or not crop-stimulating anterior lobe extracts can inhibit circulating estrone in the ovariectomized animal, particularly since Dresel (19) in the mouse and Lahr and Riddle (20) in the rat have shown that prolactin administration can temporarily suppress estrous cycles. Twenty ovariectomized mice were used to explore this possibility. Each mouse received 2 subcutaneous injections of 0.1 mg. estrone dissolved in 0.1 cc. sesame oil, separated by an interval of 24 hours. Simultaneously with each of these injections it received an injection of 0.5 mg. of the prolactin preparation in 0.1 cc. water. Vaginal smears were examined at 12-hour intervals following the last injection. Of the 20 mice, 90% showed cornified smears. Since about 95% of the mice of the colony used gave estrous smears when injected with the above dose of estrone, it may be concluded that the prolactin had caused no inhibition of circulating estrone.

An even higher ratio of crop-stimulating activity to estrone was used in another similar experiment. Though each mouse received a total dose, equivalent to 40 Riddle units (3), of a highly potent prolactin preparation together with a total of 0.2 estrone, the percentage showing cornified smears was no different from the control values. These experiments, therefore, indicate that prolactin preparations do not inhibit the action of estrone on end organs and that the anti-gonad action of crop-stimulating extracts must be due to inhibition of the secretion of follicle-stimulating hormone by the pituitary as suggested by Bates, Riddle and Lahr (13).

The effect of estrone on the body weight of pigeons. Bates, Riddle and Lahr (13) found that prolactin treatment caused an increase in body weight of adult pigeons. Simultaneous injections of low doses of estrone had no effect on the body weight increase due to prolactin. Folley and White (11), however, found that simultaneous administration of prolactin and high doses of estradiol monobenzoate caused loss in body weight, while control pigeons receiving prolactin alone gained in body weight. In these experiments administration of estrone at high doses caused loss in body weight as compared with controls. The results are given in table 2.

It is seen that in every experiment the birds receiving estrone either lost more weight than the controls, or else the former lost weight while the controls gained, or else the estrone-injected birds gained less weight than the controls. The greatest net differences between the weight changes of the estrone-injected and control birds are to be found in the series where 1.5 mg. of the prolactin preparation was injected daily, as would be expected if crop stimulating extracts, in accordance with the claims of Bates et al. (13), actually tend to cause an increase in body weight while estrone exerts the opposite tendency. This conclusion is further supported by the results of an experiment in which 10 birds received 6 daily injections of 1 mg. estrone in 1 cc. sesame oil. In these birds the mean change in body weight during the experiment was −7.4 gm.

TABLE 2. MEAN BODY-WEIGHT CHANGES IN GROUPS OF PIGEONS DURING ADMINISTRATION OF ESTRONE AND PROLACTIN[1]

Daily dose of estrone mg.	(A) Change in weight of pigeons receiving estrone and prolactin	(B) Change in weight of control pigeons receiving sesame oil and prolactin	B–A
	(a) *1.5 mg. prolactin daily for 6 days. Daily oil volume=1.0 cc.*		
0.20	+ 3.6	+17.0	+13.4
0.50	−10.0	+ 8.8	+18.8
1.00	−10.7	+ 8.8	+19.5
2.00	−20.7	+ 8.8	+29.5
	(b) *3.0 mg. prolactin daily for 6 days. Daily oil volume=1.0 cc.*		
0.75	+12.4	+26.7	+14.3
1.50	+ 6.3	+17.6	+11.3
2.00	+22.1	+39.7	+17.6
	(c) *3.0 mg. prolactin daily for 6 days. Daily oil volume=0.5 cc.*		
0.10	− 6.1	− 3.5	+ 2.6
0.50	−21.3	−18.3	+ 3.0
1.00	− 4.4	+ 3.6	+ 8.0

[1] The birds were weighed about 2 days before injections began and again 24 hours after the last injection. All body-weight changes in gm.

SUMMARY

The inhibiting effect of various doses of estrone on the response of the pigeon crop gland to prolactin has been studied.

With a given level of prolactin the absolute amount of crop growth prevented by estrone administration rose to a maximum with increasing doses of estrone which was higher when the daily dose of prolactin was 3 mg. than when the daily dose was 1.5 mg.

The percentage inhibition due to estrone administration also rose to a maximum with increasing doses of estrone but the maximum was the same for both levels of prolactin, and for the preparation used was equal to about 54%.

The power of inhibiting the crop-stimulating activity of prolactin appears to be a characteristic property of estrogenic hormones and suitable conditions for comparing the potency of other substances with that of estrone are defined.

Relatively high doses of crop-stimulating extracts of anterior lobe do not inhibit the vaginal response to estrone in the ovariectomized mouse.

Estrone and prolactin exert antagonistic effects on the body weight of the pigeon, prolactin tending to increase the body weight while estrone exerts the opposite tendency.

I wish to express my sincere thanks to Dr. A. S. Parkes and Mr. C. W. Emmens for carrying out the experiments on ovariectomized mice. My best thanks are also due to Dr. A. N. Macbeth of Organon Laboratories and Dr. K. Miescher of Messrs. Ciba Ltd. for generous supplies of estrone. The technical assistance of Mr. S. C. Watson was greatly appreciated.

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THE INHIBITION OF THE ACTION OF THE FOLLICLE STIMULATING HORMONE BY THE PITUITARY[1]

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WE HAVE REPORTED that the injection of an estrogen (amniotin) into immature female rats for 3 days previous to treatment with the follicle stimulating hormone (FSH), resulted in an increased ovarian response to the FSH and marked luteinization; this was in contrast with the purely follicular response to FSH in rats without estrogen treatment (1). The ovaries of hypophysectomized rats, similarily treated with estrogen and FSH, showed no augmentation or luteinization so the obvious conclusion was that the effect of the estrogen was on the pituitary, stimulating it to secrete LH. However, if the treatment with the estrogen was continued for 8 days, the ovarian response to subsequent FSH treatment was decreased rather than increased. We were at a loss to explain this inhibition except on the possible basis of secretory exhaustion but Evans et al. (2) have suggested that it might be due to an increased secretion of the 'antagonist' which they have reported separating from the other gonadotropic hormones of the pituitary. The function of the 'antagonist,' as described by them, is the inhibition of the ovarian response to FSH.

EXPERIMENTAL

Since estrogen produced secretion of LH from the pituitary when injected for 3 days and therefore might continue to do so for longer periods, we carried out experiments to determine if the LH preparations would produce the same inhibition as the estrogen when given to the rats for 8 days previous to the administration of the FSH. Twenty-one-day-old female rats, in groups of 6, were accordingly given various amounts of LH for 8 days and then treated with FSH for the 3 succeeding days. Both FSH and LH were injected twice daily. It was found that the response of the ovaries to the FSH was decreased after LH treatment in much the same manner as when the rats were pretreated with estrogen. Moreover, in contrast with estrogen, LH produced inhibition in hypophysectomized rats as well as in the normal animals (table 1).

It seems therefore that injection of estrogen for 8 days stimulates the pituitary to secrete something which is responsible for the inhibition of FSH action. This same substance is present in the LH preparations and produces the same effect when given directly for 8 days.

[1] Aided by a grant from the Rockefeller Foundation administered by Frederick L. Hisaw.

TABLE 1. EFFECT OF 8 DAYS PRETREATMENT OF RATS WITH LH ON THE OVARIAN RESPONSE TO FSH[1]

Total dosage of LH (mg.)	0	0.5	1.0	2.0	4.0	6.0
Ovarian weights (mg.) (a)	35	36	33	24	16	14
(b)	22			10	8	7

[1] The dosage of FSH was constant.
(a) Normal rats.
(b) Hypophysectomized rats.

The similarity of the action of estrogen and the LH preparations is also found after short periods of treatment. We have shown that augmentation of FSH action and luteinization results after 3 days of estrogen treatment (1). This is strictly comparable to the simultaneous injection of FSH and LH, since Hohlweg (3) has shown that a period of at least 2 days is necessary, following estrogen injection, before LH is secreted by the pituitary.

It is evident that the effects of estrogen and of LH extracts are the same and that augmentation or inhibition can be produced depending on the length of treatment. The only difference in the action of the two is that the LH preparations produce these effects directly while estrogen produces the secretion of the same substance, contained in the LH preparations, by the pituitary.

It has been shown that certain pituitary preparations, including pituitary LH, when given intraperitoneally, inhibit the action of subcutaneously administered FSH (2, 4, 5, 6, 7). Table 2 presents comparative results obtained when FSH and LH were injected simultaneously, the LH being injected subcutaneously in one series of rats and intraperitoneally in another, while the FSH was administered subcutaneously in all instances. It is clear that opposite effects were obtained with the same preparation depending on the method of administration. The ovaries of all the rats were luteinized to various degrees, irrespective of whether augmentation or inhibition had taken place.

TABLE 2. SUBCUTANEOUS AND INTRAPERITONEAL INJECTIONS OF LH, SIMULTANEOUSLY WITH SUBCUTANEOUS INJECTIONS OF FSH[1]

	Ovarian weights				
Total dosage of LH (mg.)	00	0.2	0.4	1.0	2.0
Intraperitoneally	38	34	26	17	18
Subcutaneously	38	62	84	87	51

[1] Dosage of FSH remained constant.

Two explanations of the inhibiting effect of the LH preparations seemed possible; either the injected material affects the ovaries so as to make them refractory or the presence of some factor in the circulation prevents the FSH from acting. In an attempt to determine which of these explanations was valid, ovaries from normal rats and also from animals which had been treated with estrogen for 8 days were implanted into normal animals and also into others which had been treated with estrogen for the same period. The ovaries were removed from the donors and a single ovary immediately implanted

under the capsule of one of the kidneys of each of the recipients. The recipients were castrated at the time of implantation. Four groups of rats were thus obtained: *a*) Treated rats with ovaries from treated rats. *b*) Treated rats with ovaries from untreated animals. *c*) Untreated rats with ovaries from treated rats. *d*) Untreated rats with ovaries from untreated rats.

The experimental animals, thus prepared, were immediately injected for 3 days with FSH. The single ovary was then dissected from the kidney and weighed to determine the degree of response. As is seen in table 3, the ovaries from 2 of the groups responded, namely estrogen treated ovaries in normal recipients and normal ovaries in normal recipients, whereas the ovaries implanted in treated recipients did not respond, irrespective of whether they were taken from treated or normal animals. It appears therefore, that the ovaries are not affected by the estrogen treatment but the bodily environment is altered and it determines whether or not the ovaries will respond to FSH. Apparently something in the circulation inhibits the FSH action.

TABLE 3. RESPONSE TO FSH OF NORMAL OVARIES AND OVARIES FROM RATS TREATED WITH ESTROGEN FOR 3 AND 8 DAYS WHEN IMPLANTED IN THE KIDNEY OF NORMAL RATS AND RATS WHICH HAD ALSO BEEN TREATED WITH ESTROGEN FOR A SIMILAR PERIOD

	Ovary weights[1] (mg.)			
Length of treatment	Group a	Group b	Group c	Group d
8 days	5.5	6.0	12.0	11.0
3 days	12.0	11.0	13.0	10.5

Group a. Estrogen treated ovaries in estrogen treated rats.
Group b. Normal ovaries in estrogen treated rats.
Group c. Estrogen treated ovaries in normal rats.
Group d. Normal ovaries in normal rats.

[1] Each figure is the average weight of single implanted ovaries from 18 animals.

Similar experiments carried out after 3 days of estrogen treatment showed that all the ovaries responded equally well irrespective of the donor or the host (table 3). It was not possible to demonstrate the augmentation in ovarian weights as was previously obtained in the normal intact animal (1) but neither was there any inhibition of FSH as was the case after 8 days of estrogen treatment.

An obvious criticism of the experiments is that the short time intervening between implantation and removal of the transplanted ovaries was not sufficiently long for the establishment of an adequate blood supply, so the responses were not as great as might be desired. However, the results were unmistakably consistent and the weight increases of the ovaries which responded were approximately 100% while those which did not respond were no different in weight from the controls.

To identify, if possible, the inhibiting substance in the blood after an 8-day estrogen treatment, 70 rats, 35 days old were treated for 8 days with 1 R.U. of an estrogen (amniotin) daily. At the end of this period, the rats

were bled and the serum from the combined blood was extracted for LH. The extract was purified so that 1 cc. of the preparation was equivalent to 20 cc. of serum. The experiment was twice repeated. The blood serum from untreated rats and from rats treated for 3 days served as control material. The preparations were tested in 4 ways: *a*) They were injected into immature male rats to determine the presence of luteinizing hormone. *b*) They were given simultaneously with but separately from FSH, both preparations being given subcutaneously, also to detect LH by the augmentation and luteinization. *c*) They were tested on immature female rats to detect FSH. *d*) They were given intraperitoneally in female rats while FSH was injected subcutaneously to determine if any inhibition of the FSH would result.

The results clearly demonstrate the presence of LH in the blood serum of rats treated for 8 days in greater concentration than in that from rats treated for 3 days or the untreated animals. Twenty cc. of the serum from rats treated for 8 days produced interstitial cell stimulation in the male and when injected subcutaneously with FSH, augmentation and luteinization in the female. Injected intraperitoneally while FSH was administered subcutaneously the 8-day serum produced some inhibition which was not however very marked. No FSH was demonstrated in the serum. The only positive response of the normal serum was slight augmentation without luteinization when given subcutaneously with FSH.

TABLE 4. ASSAY OF BLOOD SERUM FROM RATS TREATED WITH ESTROGEN FOR 3 AND 8 DAYS FOR GONADOTROPIC HORMONES

Injections	Ovarian and seminal vesicle weights (mg.)		
	8-day serum	3-day serum	normal serum
Imm. ♀ s. 20 cc. serum	11	12	10
Imm. ♂ s. 20 cc. serum	18	11	10
Imm. ♀ s. FSH alone. 2 R.U.	25		
Imm. ♀ s. 2 R.U. FSH subcut. 20 cc. serum subcut.	43	27	29
Imm. ♀ s. R.U. FSH subcut. 20 cc. serum intrap.	20	26	27

DISCUSSION

While the results which have been presented do not prove conclusively that the factor which is responsible for the antagonistic action of pituitary extracts is the luteinizing hormone, they do show that the principle is at least always closely associated with the LH. For example, LH preparations always produce inhibition when injected intraperitoneally and when inhibition is not complete the ovaries are luteinized. In the same manner, LH preparations have an inhibiting and luteinizing action when injected for 8 days previous to FSH treatment, and further, after inhibition has been produced by estrogen treatment, the blood of the animals gives unmistakable evidence that LH is present in higher concentration than after 3 days of such treatment. After 3 days of estrogen treatment, LH is being secreted by the pituitary as shown in our previous work, but the concentration at that time is

apparently not high enough to be recovered by the methods used. Inhibition of FSH after 8 days and augmentation after 3 days of estrogen treatment are correlated with these facts.

Following the report of Evans and coworkers (2) that they had separated the 'antagonist' from the follicle stimulating and the luteinizing hormones of the pituitary and had found it to be a separate principle, we attempted to repeat their work without success. The antagonistic action was always exhibited by the preparations containing the luteinizing hormone and we have not been able to obtain any indication of a separate inhibiting factor distinct from LH by the methods of Evans or by other methods. At the present time it seems that the antagonistic action may be a characteristic of the luteinizing hormone. How the effect is brought about is obscure but it seems that the concentration of LH in the blood may be the determining factor and when high, as compared to that of FSH, inhibition is produced. If the LH is absorbed more rapidly than the FSH, as is probably true when the LH is injected intraperitoneally and the FSH is given subcutaneously, this concentration may be reached and inhibition of the FSH results.

It appears that estrogen actually causes both production and release of LH from the rat pituitary. The pituitary of an immature rat evidently does not normally secrete much LH nor is its LH content high enough to produce the effects here described, which makes it impossible to account for the results by the elimination of the LH already present.

The fact that estrogen apparently stimulates the rat pituitary to continuous secretion of LH in large enough amounts to cause inhibition of the follicle stimulating hormone may be of significance in an explanation of the estrous cycle of the rat. Our previous explanation of the estrous cycle was based on the fact that estrogen increases the secretion of LH and decreases the secretion of FSH. This same process would be still more effective in concluding the follicular phase of the cycle if the LH secreted, due to estrogen stimulation, not only luteinized the follicles but also inhibited the FSH action on the ovaries. Under these conditions the pituitary would not have to stop secreting FSH as the LH would render it inactive and thereby effect a rather sharp transformation from FSH to LH action.

SUMMARY

Estrogen and luteinizing hormone preparations inhibit the action of FSH on the ovaries of immature rats when injected for 8 days previous to FSH treatment. In hypophysectomized immature rats, estrogen does not produce this inhibition whereas the LH are as active as in normal rats. Estrogen therefore produces the effect by stimulating the pituitary to secrete the same substance which is administered to the rats in the LH preparations.

The inhibiting substance does not produce inhibition by direct action on the ovaries making them refractory to FSH since the ovaries from rats which had been treated with estrogen for 8 days responded to FSH when implanted into animals which had not been treated with estrogen. On the

other hand, ovaries from normal rats implanted into treated rats did not respond, showing that the bodily environment determined the responsiveness of the ovaries.

Assay of the blood of rats treated with estrogen for 8 days showed that LH was present in greater concentration than in normal rats or in rats which had received estrogen for only 3 days. Correlated with this fact is the augmentation of the FSH action after 3 days and inhibition of FSH action after 8 days of estrogen treatment.

Attempts to separate the antagonistic principle from the luteinizing hormone were unsuccessful. It seems that the inhibiting agent is either the LH or a factor which is closely associated with and very similar to it.

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EFFECT OF LOCAL APPLICATION OF PROGESTERONE ON THE RABBIT UTERUS

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CHARACTERISTIC effects of injection of many of the hormones may be obtained by local application to responsive tissues, usually in smaller doses than those required to produce the same effect when injected in the usual way.

Berger (1) obtained vaginal smears characteristic of estrus in castrated adult rats with 1/12 of the minimal subcutaneous dose of estrogen. The hormone in suitably diluted solution was applied by means of a small ureteral catheter in 0.05 cc. quantities, 5 times during a period of 36 hours. Berger has reported also (2) that uterine stimulation and growth takes place with vaginally applied estrogen, and with lesser quantities than those required to induce the same degree of growth when given subcutaneously.

Lyons and Templeton (3) reported even more striking results than did Berger. They induced cornification of the vaginal epithelium in rats with 1/200 of the subcutaneous unit, and were able to detect estrin in 0.1 to 0.8 cc. of normal woman's urine. No uterine effects were evident.

Growth of the nipples of male guinea pigs which may be induced by estrin injections may also be obtained by liquid or ointment solution applied directly to the nipples (4). Selected amounts of hormone which have no effect on the untreated control nipple produce maximal growth on the treated side. From the experimental data given it was not possible to compare local with subcutaneous minimal requirements for nipple effects.

Numerous investigators have demonstrated that comb growth of the capon or chick is produced by injection of androgenic substance directly into the comb or merely by application (in solution) to the surface of the comb. The inunction method of administering male hormone is said to be 50 times as sensitive as the intramuscular.

Lacassagne and Raynaud (5) introduced testosterone into the lumen of the seminal vesicles of adult rats which had been castrated 20 days previously. The volume of olive oil solution of hormone was 1/40 cc. They reported that a single injection of 2.5 γ of testosterone caused unmistakable epithelial growth on the fourth day after injection.

The present report demonstrates that progesterone, in single doses of 0.5 to 5 γ introduced into isolated loops of the uterus of immature rabbits previously treated with theelin, will produce progestational proliferation equivalent to that brought about by a total of 0.5 mg. given intramuscularly in 5 daily divided doses. These results are quite contrary to those of de-Fremery, Luchs and Tausk (6) who reported no progestational changes with one-third rabbit unit progestin placed in a uterine horn.

EXPERIMENTAL METHODS

Rabbits of 950 gm. average weight after preliminary treatment with theelin, 150 I.U. over 6 days, were anesthetized with ether, the abdomen opened and the uterus exposed. An upper middle segment of each horn of 3 to 4 cm. in length was selected and ligatures placed at each end. The upper ligature was tied tightly, the lower being left loose, both being so placed so as to avoid disturbing the blood supply to the segment.

Each horn was sectioned below the loose ligature. Crystalline progesterone dissolved in 0.1 cc. peanut oil or lanolin was introduced into the lumen of the isolated segment using a small syringe provided with a blunted needle. As the contents of the syringe was forced into the uterine cavity and the needle withdrawn, the ligature was tightened. The same volume (0.1 cc.) of oil or of lanolin without hormone was placed into the corresponding uterine segment on the opposite horn. The abdomen was closed. In 3 experiments ovariectomy was done at the time of introduction of progesterone.

The rabbits were killed at 72 hours. One or two portions of each horn below the isolated loop as well as from the injected segments on each side were removed for histological examination. The degree of reaction is expressed according to the McPhail scale (7).

RESULTS AND DISCUSSION

In no case did any portion of uterus in the control (oil- or lanolin-injected) segments, or in the uninjected regions on either side below the tied-off loops, show any trace of progestational proliferation.

In the table are listed the results of application of progesterone in single doses ranging from 0.02 to 0.000125 mg. in 2 series, the first in peanut oil solution and the second in lanolin.

It will be noted that while there is considerable variation in response among the different animals at the same dose, there is a fairly uniform diminution in reaction with decreasing amounts of hormone and good parallelism between the use of peanut oil and lanolin. Here it should be pointed out that no attempt other than the use of two solvents has been made to determine the optimal condition for maximal reaction by this method. It would be necessary to undertake further study in order to place the method on a quantitative basis.

It has been our experience when assaying progesterone using the McPhail procedure, that 0.5 mg. of progesterone causes a +2 or +3 reaction in the majority of rabbits weighing not over 1200 gm. nor less than 800 gm.

Inspection of the table shows that out of 22 animals receiving 0.0005 to 0.005 mg. of progesterone in a single local dose, 20 gave a response equivalent to that of 0.5 mg. by the multiple intramuscular injection method in the intact animal. Of the 12 rabbits receiving 0.01 and 0.02 mg. progesterone the response in 9 of this series was probably maximal under the circumstances of the experiment. We have no explanation for the lesser reactions in the other animals of this group.

In 10 rabbits local application of 0.25 and 0.12 γ in no case failed to show an entirely negative reaction, but it is evident that this quantity of hormone is near the minimum.

TABLE 1. INTRAUTERINE APPLICATION OF PROGESTERONE. EXAMINATION AT 72 HOURS

0.1 cc. Peanut oil			0.1 cc. Lanolin		
Rabbit wt.	Progesterone	Reaction	Rabbit wt.	Progesterone	Reaction
	mg.			*mg.*	
860	.02	3–4	1040	.02	4
1060	.02	3–4	1060	.02	3
1000	.01	3	1060	.01	3–4
1000	.01	1–2	1000	.01	4
1300[1]	.01	2, 2			
1220[1]	.01	3, 4			
1400[1]	.01	3–4, 3–4			
1460	.01	0–1, 1			
920	.005	2	960	.005	3
740	.005	3	900	.005	2
1040	.005	2, 3	920	.005	4, 4
1100	.005	2, 2	600	.005	4, 2–3
790	.001	2	900	.001	1
840	.001	2	740	.001	2
940	.001	2–3	940	.001	3–4, 3–4
1000	.001	2, 2–3	800	.001	2, 1
			800	.001	1–2, 2
860	.0005	2–3, 2	740	.0005	2, 2–3
1180	.0005	1–2, 1	780	.0005	2, 2
			660	.0005	1, 2–3
1280	.00025	0, 1	720	.00025	1, 2
1280	.00025	1, 1	620	.00025	0–1, 0–1
1320	.00025	1, 1	600	.00025	0–1, 0–1
1040	.00025	0–1, 1			
1200	.00025	1, 1			
1060	.000125	1–2, 1–2			
900	.000125	1, 1			

[1] Bilateral ovariectomy at time of intrauterine application of progesterone.

In view of the previously published report of deFremery, Luchs and Tausk, the results are surprising in that such small amounts of locally applied progesterone produce progestational reactions. On the other hand physical conditions for optimal effect of the hormone on the uterine epithelium are present; the progesterone was in direct contact with the cell, with minimal opportunity of being removed from the area. While it is not possible to calculate the concentration of progesterone administered intramuscularly within or at the cell surface of the uterine epithelium, except in a rough way, the value is probably less than that which exists under the conditions of this experiment.

No evidence of 'decidual reactions' were noted. It was not likely, however, that such reactions would be apparent in the 72-hour interval, despite

the possibility of irritation stimuli from the oil or lanolin. Bilateral ovariectomy in 3 animals did not modify the results.

SUMMARY

Crystalline progesterone introduced directly within the lumen of theelin-sensitized immature rabbits produced characteristic progestational proliferation of the endometrium. Single applications of 0.0005 to 0.005 mg. in 0.1 cc. of peanut oil or lanolin in segments of uterus 3 to 4 cm. in length caused reactions in 72 hours equivalent to those obtained with 0.5 mg. progesterone administered in divided doses intramuscularly in the intact rabbit. Application of 0.000125 to 0.00025 mg. produced threshold endometrial reactions.

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EXCRETION OF URINARY ANDROGENS FOLLOWING THE INJECTION OF TESTOSTERONE PROPIONATE

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THIS PAPER presents the results of 121 assays for urinary androgens in 16 cases of hypogonadism in men and in 6 normal men. The method employed for assay of the urine of the patients was that of Gallagher and Koch (1, 2), while in the normal group the method of McCullagh and McLin (3) was used. In each assay 5 birds were employed and 5 controls were injected with androsterone. The normal range lies between 18 and 90 I.U. per 24-hour specimen of urine. In normals, 22 assays by the 1st method average 39.3 U and 26 assays by the 2nd averaged 35.7 U. The probable error in the 1st group is 6.52 and in the 2nd is 5.03.

Group 1. Testosterone propionate[1] in doses varying from 5 to 50 mg. was given to hypogonadal individuals and the urinary androgens during the succeeding 24 hours were measured. Assays done in this group preceding treatment all gave findings below the normal range, the average being 4 U.

Seven assays were done following 5 mg. doses in 4 cases. The 24-hour excretion following this averaged 24 I.U., the extremes being 13 and 34. Fourteen assays were done on 24-hour specimens of urine following 10 mg. injections. These averaged 26 I.U. The range was 11 to 94 U, only one assay being above 35. Five assays, in 5 cases, following 15 mg. doses averaged 57 U. Four assays, in 4 cases, following 20 mg. doses averaged 57 I.U. Eleven assays, in 9 cases, following 25 mg. doses averaged 63 U. Four assays, in 2 cases, following 50 mg. doses averaged 68 U.

Table 1 shows the details of the assays in this group of cases. The form in which these androgens are excreted is not known. For purposes of comparison, however, the excreted androgens have been recalculated as milligrams of androsterone and the average excretions in the various groups mentioned are shown graphically in figure 1.

From these assays it appears that the amount of androgen excreted during 24 hours following the injection of testosterone propionate is to some degree proportionate to the amount of the drug administered. In these cases 4 assays on urine collected on the 2nd day following 5 and 10 mg. doses showed between 1 and 7 U. Three assays on urine collected the 2nd day following 20 and 25 mg. doses yielded 30, 35, and 40 U respectively.

[1] The testosterone propionate used in these studies was oreton supplied through the courtesy of Dr. Erwin Schwenk, Dr. Max Gilbert, and the Schering Corporation.

TABLE I

Case No.	Age	Assays before treatment	Assays during 24 hours following injection of testosterone propionate					
			Dosage in mg.					
			5	10	15	20	25	50
1	38		20	17	64	37	104 41	
2	33	0	32 15 13	35				
3	32	1 2		24 19				
4	26	2	28 28		47		115	
5	50	2 0	34		103			
6	20	0		31 31			38	
7	29	3 2 4		94	38		96	60 59
8	51	3 5 1					65	
9	32	2				74	40 43	
10	22	16		24		65		
11	63	7				52		
12	52	7		18				
13	33	2		11 16			36	
14	65	0		18 17 16	35			
15		16					78	
16		8					42	68 86
Averages		4	24	26	57	57	63	68

In single cases, repeated assays were done on 24-hour specimens collected immediately following various doses of hormone. A general parallelism of dose and excretion can be seen, though the irregularity is considerable. Case one is a good example of this, the assays following various doses being as follows: 5 mg., 20 I.U.; 10 mg., 17 I.U.; 15 mg., 64 I.U.; 20 mg., 37 I.U.; and 25 mg., 104 I.U. It is seen that the assay following a 25 mg. dose is about 5

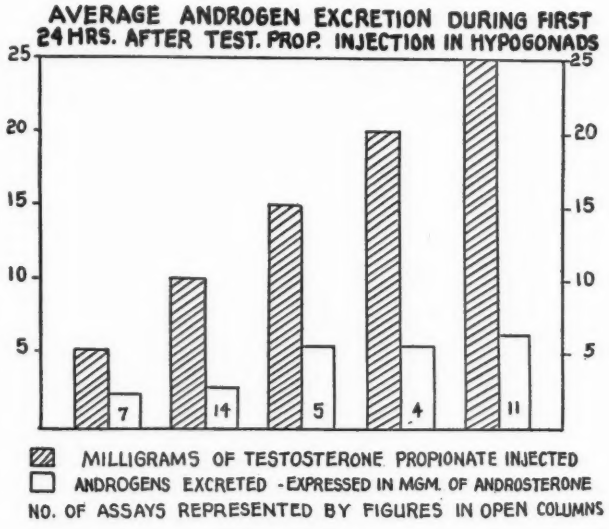
AVERAGE ANDROGEN EXCRETION DURING FIRST 24 HRS. AFTER TEST. PROP. INJECTION IN HYPOGONADS
25
20
15
10
5
7
14
5
4
11
MILLIGRAMS OF TESTOSTERONE PROPIONATE INJECTED
ANDROGENS EXCRETED - EXPRESSED IN MGM. OF ANDROSTERONE
NO. OF ASSAYS REPRESENTED BY FIGURES IN OPEN COLUMNS

Fig. 1

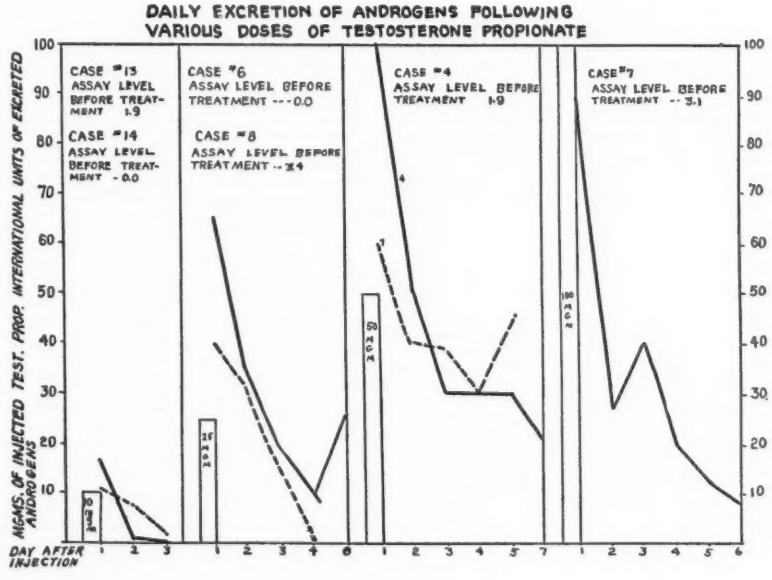
DAILY EXCRETION OF ANDROGENS FOLLOWING VARIOUS DOSES OF TESTOSTERONE PROPIONATE
CASE #13
ASSAY LEVEL BEFORE TREATMENT 1.9
CASE #14
ASSAY LEVEL BEFORE TREATMENT - 0.0
CASE #6
ASSAY LEVEL BEFORE TREATMENT --- 0.0
CASE #8
ASSAY LEVEL BEFORE TREATMENT -- 3.4
CASE #4
ASSAY LEVEL BEFORE TREATMENT 1.9
CASE #7
ASSAY LEVEL BEFORE TREATMENT -- 3.1
MGMS. OF INJECTED TEST. PROP. INTERNATIONAL UNITS OF EXCRETED ANDROGENS
DAY AFTER INJECTION

Fig. 2

times the assay following the 5 mg. dose. In some instances, as in case 5, the amount of androgen excreted is in direct proportion to the amount of testosterone propionate injected. In this instance the injection of 5 mg. of the drug is followed by excretion in 24 hours of 3.4 mg. of androgen calculated as androsterone while a 15 mg. dose results in the excretion of 10.3 mg.

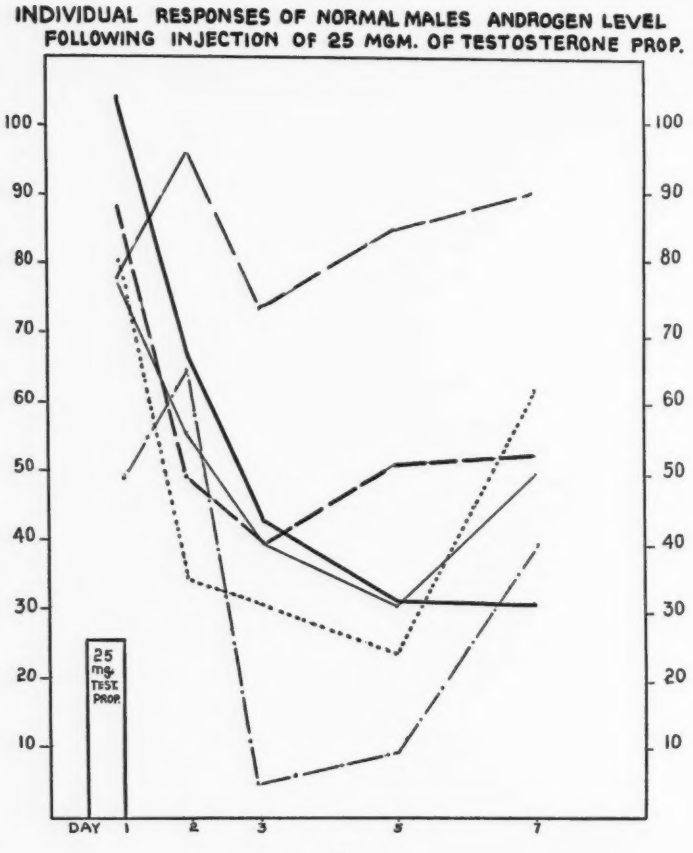

Fig. 3

Group 2. In this group the excretion of androgens was followed daily for several days after single injections in cases of hypogonadism. These results are shown in figure 2.

In two cases 10 mg. doses were used. The androgen excretion rose definitely during the first 24 hours and fell promptly to previous levels on the 2nd and 3rd day.

Following the injection of 25 mg. the urinary androgen levels remained within normal range for 2 to 3 days and were strictly within normal range for the first 2 days. Case 8, also, illustrates a tendency to a secondary rise

following a depressed level as is seen in patients in whom considerable androgen of their own is produced as in the normals above.

Following doses of 50 and 100 mg., the levels of urinary androgens rose more markedly, fell rapidly, but remained within normal range for 4 to 7 days.

It appears from these observations that in cases of frank hypogonadism to 10 mg. doses of testosterone propionate may be expected to cause a rise in urinary androgens toward but not to within normal range. Such a rise is maintained for little more than one day. Larger doses raise these levels further and maintain them within normal range for longer periods depending upon the size of the dose given. If the total quantity of androgen excreted is calculated in terms of androsterone, it appears that in these cases amounts varying between approximately 20% and 70% of the injected quantities can be accounted for.

Group 3. Each of 6 normal men were given 25 mg. of testosterone propionate. Their daily excretion of urinary androgens were measured on the following 1st, 2nd, 3rd, 5th, and 7th days. Figure 3 shows that there is under these conditions a sharp and rather marked rise in androgen excretion. This is followed by a sharp fall beginning by the 2nd day. On the 3rd to the 5th days levels of excretion are reached which are lower than the levels found in these individuals normally, and this, in turn, is followed by a rise to within normal range. There is no proven explanation for this phenomenon but from the foregoing observations a possible explanation presents itself. The body has probably excreted most of the injected androgens by the 3rd day as in the cases of hypogonadism. In the normals the initial rise in excretion is interpreted as being due to this. The depression of the urinary androgen levels which follows is believed to be due to the pituitary depression known to be a function of injected androgens. If this is the case, it appears that the pituitary escapes from this depressant effect in 6 to 7 days allowing the production and excretion of androgens at a normal rate.

SUMMARY

Following the injection of testosterone propionate in hypogonadal men there is an increase in excretion of urinary androgens. The extent of this increase is in proportion to the size of the dose.

In cases of hypogonadism, injection of 5 to 10 mg. of testosterone propionate is followed by an increase in urinary androgens for not more than about 24 hours. With larger doses the increased excretion is maintained for several days.

In normal men injection of testosterone propionate is followed by a prompt rise in excreted androgens, then for several days there is a fall and subsequently a second rise to normal levels. A possible explanation of this phenomenon is offered.

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A CASE OF ARRHENOBLASTOMA WHICH SIMULATED CUSHING'S DISEASE[1]

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LANGDON-BROWN (1) believes that Cushing's syndrome may be initiated apparently by changes either in the pituitary, thymus, adrenals or ovary. The only pathological finding common to these various origins of the syndrome is a hyaline change in the basophilic cells of the adenohypophysis which is not interpreted as degenerative, but is considered an expression of altered physiological activity. Crooke (2) who discovered this cytologic picture regards it as of fundamental importance.

Levy Simpson (3) describes the Cushing syndrome as characterized by plethoric adiposity of the face, neck and trunk, associated, in women, with hypertrichosis and amenorrhea. Other features found are hypertension, purplish striae distensae of the abdomen, acne and acrocyanosis. Hyperglycemia, with or without glycosuria, and osteoporosis are also characteristic of this syndrome, although less uniformly present.

The possibility of hyperactivity of the adrenal cortex producing basophilism of the pituitary is conceivable since in Addison's disease, the opposite, a decrease in the basophil cells, is usually found.

Taking up the clinical features in greater detail, we find that the menstrual periods occur at increasing intervals and become scanty; finally amenorrhea is established. The adiposity of the face, neck and trunk develops rapidly, sparing the limbs—a 'buffalo type' of distribution. The hair of the head may fall out, especially over the vertex and in the temporo-frontal regions, receding as in the male. Hair grows on the upper lip, the beard region, thighs, legs, forearms, chest and abdomen, with a masculine pubic escutcheon. Hypertrichosis is not always excessive; in fact, hirsutism of a marked degree would incline one to give first consideration to disease of the adrenal cortex. With the rapidly developing adiposity, lineae distensae develop in the lower abdomen, mammary and axillary regions; these are usually dusky red or purple rather than white. Polyphagia, polydipsia and polyuria are frequently present. Occasionally there is pigmentation of the skin of the orbital region, axillae and nipples. The plethoric cyanotic appearance, which is characteristic, is occasionally associated with a polycythemia. Both the systolic and diastolic blood pressure levels are elevated. Headaches are the result of the increased blood pressure and are not due to pressure of the adenoma within the sella. Glycosuria and hyperglycemia arise in many cases, like diabetes associated with acromegaly; this condition is slowly progressive, shows

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periods of remission, is refractory to insulin and may respond to x-ray treatment directed to the pituitary. Bones becomes decalcified and soft; kyphosis develops as a result of the weight of the body producing a constant compression of the weakened vertebrae. Blood calcium is not elevated but calcium may be excreted in excessive amounts. The thyroid is sometimes enlarged but the basal metabolic rate is rarely increased; nevertheless exophthalmos is often found. Amenorrhea and sterility, with

Fig. 1, 2, 3. *Mrs. M. D'A.* MARKED HYPERTRICHOSIS OF FACE, CHEST, ABDOMEN, BACK, ARMS AND LEGS. OBESITY WHICH WAS RAPIDLY ACQUIRED. Fig. 4, 5. PATIENT BEFORE OPERATION. NOTE HEAVY GROWTH OF HAIR ON FACE WITH TYPICAL 'MUTTON CHOPS.' Fig. 6. PATIENT 6 MONTHS AFTER PITUITARY OPERATION. NOTE DEFINITE DECREASE IN HAIR ON FACE WITH MARKED FRAYING OUT OF 'MUTTON CHOPS.'

small atrophic ovaries, is the usual finding; however, there are proven cases that had normal menstrual periods and multiple pregnancies. The clitoris may be normal or markedly hypertrophied. Considerable enlargement of the clitoris is more apt to be associated with primary adrenal cortex lesions and arrhenoblastoma of an ovary.

In the differential diagnosis one must rule out ovarian tumors, disease of the adrenal cortex and, perhaps rarely, oat-cell tumor of the thymus. Thymic tumors may

Fig. 7. X-RAY OF SKULL SHOWING SLIGHTLY ENLARGED SELLA—DORSUM OF SELLA IS QUITE THIN, ALSO SHOWING SLIGHT DEMINERALIZATION OF BONES OF SKULL.

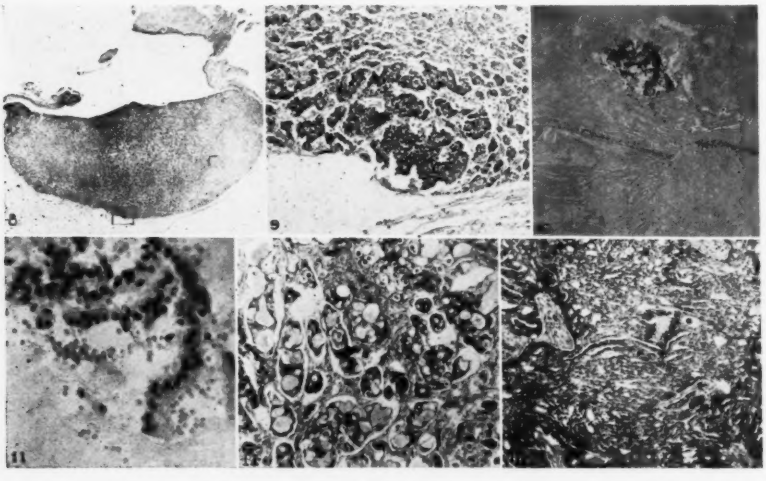

Fig. 8. PITUITARY GLAND. NOTE LARGE CYST PARTIALLY LINED BY A SINGLE LAYER OF CUBOIDAL EPITHELIUM. Fig. 9. HIGH POWER VIEW OF SECTION OF PITUITARY SHOWING ADENOMATOID HYPERPLASIA OF BASOPHILS, NOT ENCAPSULATED; NO HYALIN CHANGES FOUND IN THE BASOPHILS. Fig. 10, 11. TISSUE REMOVED AT PITUITARY OPERATION CONSISTING OF FIBROUS TISSUE AND ATROPHIC PITUITARY CELLS. Fig. 12. SECTION OF TUMOR MASS IN PELVIS SHOWING DEFINITE TUBULAR FORMATION WITH SOME AMORPHOUS COLLOID-LIKE SUBSTANCE IN THE LUMEN. Fig. 13. SECTION OF TUMOR TISSUE FROM LIVER REGION SHOWING CUBOIDAL CELLS IN DEFINITE TUBULAR FORMATION.

be identified sometimes by x-ray examination. Ovarian tumors, namely arrhenoblastoma, rarely cause this syndrome, only 39 cases having been reported in the literature, and some of these, according to Norris (4) are doubtful. Enlargement of the sella is extremely rare, but if found would favor a diagnosis of a pituitary lesion (Cushing's disease). If the clitoris is considerably enlarged and there is greasy skin with acne, rather than dry skin, the diagnosis would favor the adrenal syndrome even in the face of normal pyelograms and normal perirenal air studies. The adrenogenital syndrome may be produced by benign adenoma, adrenal cortical hyperplasia or malignant hypernephroma. The syndrome will develop rapidly if caused by cortical carcinoma and slowly if caused by a benign lesion of the adrenal cortex. Its characteristic features are masculine hirsutism of pronounced degree, obesity, amenorrhea, enlargement of the clitoris, atrophy of the breasts, and perhaps deepening of the voice; hypertension, acne, striae and glycosuria are not as constant findings as in Cushing's disease. In this connection, however, attention should be drawn to the Achard-Thiers syndrome of adrenal cortical origin, namely the diabetes of 'bearded women.'

Arrhenoblastoma is a rare ovarian tumor which produces defeminization and masculinization. The first report to appear in the literature was published by Pick in 1905 (5). In 1936, Baldwin and Gafford (6) reviewed the cases published up to that date and contributed the 34th case. Since that time Maxwell (7) added another classical case. This patient was originally seen and diagnosed preoperatively by one of us (H.L.). Other cases have been reported by Phelan (8), Depuy (1937) and Norris (4).

There is a close relationship between the adrenal cortex and the gonads, for both have a common embryological origin. Both glands may produce masculinization of the female. Adrenal cortical tumors may be found in the adrenal itself, or in accessory tissue anywhere along the primitive urogenital fold. Cases of adrenal cortical virilism have revealed the offending tissue to be present in the broad ligament, the retroperitoneal space and even in the ovary.

Meyer (9) in 1931 classified arrhenoblastoma under three divisions: *a*), the adenoma tubulare testiculare, composed of the mature forms and the partially carcinomatous forms; *b*), the middle group with typical and atypical tubular as well as solid forms; *c*), the atypical group composed mostly of solid forms with atypical tubular portions.

Patients with tumors of the first type show no masculinization or defeminization. The third group show the most pronounced masculine changes, whereas the second group is intermediate both clinically and histologically.

A typical history in a case belonging to the third group is one of normal maturity followed by signs of defeminization, such as loss of normal feminine curves, atrophy of the breasts, thinning out of the hair of the head, masculine pubic escutcheon, growth of beard, growth of clitoris, deepening of the voice, and profuse menstruation followed by amenorrhea but without decrease in libido.

Baldwin's case (6) showed hirsutism, falling out of the hair of the head, growth of beard, pubic hair which covered the whole abdomen, atrophic

breasts, enlarged clitoris, normal blood pressure and normal pyelograms. A diagnosis of arrhenoblastoma of the third group was made. Following surgical removal of the tumor the patient made a gradual return to normal feminism.

Maxwell's case (7) showed a very pronounced masculine hirsutism involving almost the entire body including the back and shoulders, falling of scalp hair in the temporo-frontal region, growth of beard, masculine pubic escutcheon, loss of feminine contour, atrophic breasts, enlarged clitoris, deepened voice, hypertension, polycythemia, diabetic glucose tolerance curve, B.M.R. of +39%, normal sella and normal pyelograms. Diagnosed as arrhenoblastoma, the patient was gradually restored to normal following surgical removal of the tumor.

Attention should be called to the case reported by Phelan (8) which showed defeminization and masculinization and was restored to normal following surgical removal of an arrhenoblastoma. The restoration was most conclusive for 2 months after her operation she began to menstruate, and 4 months postoperatively she became pregnant. Her baby was born at term and was successfully nursed by the patient. Of great importance is the fact that the arrhenoblastoma was of type one, according to Meyer's classification. According to Meyer, this type is not supposed to produce masculinization. Goodall (10) in discussing Phelan's case, calls attention to the fact that in his own case, although his patient had an arrhenoblastoma of the third type, she showed no evidence of either defeminization or masculinization. Goodall therefore concludes that on the basis of these two cases Robert Meyer's artificial subdivision, as to the presence or absence of sex change, completely collapses.

REPORT OF CASE

We herewith add another case to the as yet not too voluminous literature. This report seems justified, since the case is not entirely typical, and during the 4-year observation of this patient some interesting clinical and pathological data were accumulated.

Mrs. M. D'A., a married woman aged 29 years complained of obesity, amenorrhea, headaches, and hypertrichosis. The patient is 61 inches tall and maintained a weight of about 150 lb. until the age of 20 years. During the next 4 years she gradually gained up to 215 lb. By diet and the administration of thyroid she succeeded in losing 55 lb. during the next 6 months. The weight remained at this level until 2 years ago when she suddenly gained 40 lb. in 2 months' time. Her weight has been constant since then.

Her menstrual periods began at the age of 15 years and were regular every 28 days with a 4-day moderate flow. After the rapid gain of weight, 2 years ago, the patient had 3 normal menstrual periods, followed by amenorrhea which has persisted. She experienced neither hot flashes nor any 'cyclic' symptoms. She married at the age of 16 years and had normal marital relations up to the time of this history. There were no pregnancies, contraceptives having been used. Her husband is living and well. Six months after the last menstrual period the patient began to have dull 'pressure' headaches, which occurred daily and lasted for about 2 hours. About the same time

as she experienced this rapid gain in weight the patient became conscious of a growth of hair on the upper lip and over the whole beard region, but especially on the lateral aspects of the face. During the next 6 months she developed a heavy growth of hair on her breasts, abdomen, back and thighs.

The family history is of interest because of a sister aged 36 years, who had a similar hypertrichosis until she had a salpingo-oöphorectomy 5 years ago, following which all the excess body hair fell out.

Examination showed a markedly obese, olive-skinned woman, with a heavy beard and characteristic 'mutton chops,' and a pronounced heterosexual hypertrichosis. There was a large amount of coarse hair on both breasts and also in the mid-region of the chest. The abdomen was completely covered with hair which extended down to, and was continuous with, the pubic hair. The arms, forearms, back, thighs, legs and even the fingers and toes were heavily covered with hair. The thyroid was moderately enlarged, smooth in contour, and free from evidence of dysfunction. The heart was within normal limits of size; there were no murmurs or other irregularities. The blood pressure was 120/80. The abdomen was pendulous, soft, and not tender. The liver and spleen could not be palpated, nor were there any other palpable masses. The abdomen contained many longitudinal striae but they were silvery grayish-white in appearance. They showed neither the dusky red nor purplish coloring seen in Cushing's disease. The pelvic examination was made by the gynecology department and the clitoris, as well as the uterus, tubes and ovaries were reported to be within normal limits.

Laboratory findings showed the urine to be entirely normal. Blood and spinal Wassermann tests showed negative results. The blood examination showed: Hb. 90%; r.b.c. 4,500,000; w.b.c. 9,000 with a normal differential. The blood serum calcium was 9.9 mg. %; the B.M.R. −6%; the blood cholesterol was 256 mg. %. A glucose tolerance test gave the following values: fasting blood sugar, 83 mg. %; ½ hour after glucose, 168 mg.; 1 hour after, 222 mg.; and 2 hours after, 99 mg. %. With the Frank test, specimens examined at weekly intervals showed results which were interpreted as indicating a normal ovarian cycle.

X-ray examination showed the sella to be at the upper limits of normal, or possibly slightly enlarged in size; the dorsum sellae was quite thin. There was no evidence of bone erosion. There was a slight amount of demineralization of the bones of the skull. Roentgen findings were consistent with, but not diagnostic of, an early tumor of the pituitary. There was no evidence of demineralization in the thoracic spine. Urograms of the kidneys showed normal outlines with no distortion of the upper poles; the upper calices were normal in appearance. The chest was entirely normal and there was no evidence of a persistent thymus.

With the foregoing history and findings one must consider a diagnosis of adrenal cortex tumor or hyperplasia, basophilic adenoma of the pituitary (Cushing's disease), arrhenoblastoma of the ovary and thymic tumor. The possibility of thymic tumor was dismissed because of the negative x-ray findings and the extreme rarity of oat-cell tumor of the thymus. The hirsutism, together with amenorrhea, could be accounted for by any one of the other conditions.

In addition to the heterosexual hypertrichosis and amenorrhea there were many findings which favor the diagnosis of basophilic adenoma of the pituitary (Cushing's disease). The persistent occurrence of headaches which

were not on a hypertension basis, together with x-ray evidence of an enlarged sella, added a great deal of weight; demineralization of the bone is a cardinal feature of Cushing's disease and in this case there was slight demineralization of the bones of the skull; the findings of a diabetic type of glucose tolerance curve was of significance; the finding of a normal-sized clitoris is not inconsistent with a diagnosis of basophilic adenoma of the pituitary, whereas, on the contrary, Badwin's review of the literature on arrhenoblastoma revealed the fact that in every case in which the clitoris was reported, it was recorded as being enlarged. The extremely rapid gain of weight still further strengthened this diagnosis.

The striae were white rather than the characteristic dusky red or purplish color. There was no increase in hemoglobin or number of red blood corpuscles, although that is not an essential finding. The normal blood pressure is definitely contrary to the finding in Cushing's disease.

Directing our attention away from the diagnosis of arrhenoblastoma were the following pertinent facts. Pelvic examination by a competent gynecologist failed to reveal any abnormalities; the Frank test showed normal findings, indicating a normal ovarian cycle: the normal-pitched voice and persistent feminine curves are contrary to the reported essential findings.

Inconsistent with a diagnosis of disease of the adrenal cortex were the findings of a normal clitoris and negative urograms. It is admitted, of course, that abnormal urograms with distortion of the upper calices in one or both kidneys will be found only when an adrenal cortex tumor is sufficiently large to encroach upon the upper pole of the kidney to a considerable degree. Peri-renal air injections for outline of the adrenals had not been instituted when we first saw this patient.

Since the preponderance of evidence was in favor of a diagnosis of basophilic adenoma of the pituitary, and since there was x-ray evidence of enlargement of the sella, it was decided to enlist the aid of the neuro-surgical department. They concurred in the possibility of pituitary tumor and decided to attempt its removal surgically. We were encouraged in this decision by the remarkable improvement following partial hypophysectomy in a typical case of Cushing's disease.

A trans-frontal approach to the pituitary was used and the sella exposed. To the surprise of the neuro-surgeon the sella appeared empty except for a small fragment of tissue in the bottom of the cavity. The tissue was of a slightly yellowish color, and no larger than the head of an ordinary match (3 or 4 mm.). This was removed by a special tiny curette. The pathologist reported the specimen to be composed of fibrous tissue and atrophic pituitary cells. The patient made an uneventful recovery and left the hospital two weeks later.

The day following the operation the patient remarked that her head felt unusually clear and she was free from headache for the first time in many months. In order to be able properly to evaluate any changes that might take place in this patient, she was advised to make no changes in her diet or mode of life and was warned to take no medications of any nature, and per-

suaded not to employ any depilatory measures. Shortly afterwards we were surprised to observe that the hair on the face became brittle and would break off when the patient wiped her face. This change was confined to the hair on the face. Following the operation the patient also lost her sense of smell; this did not return.

At the end of a 6-month period there was a marked decrease in the amount of hair on her face; there was a striking 'fraying out' of the 'mutton chops'; the body hair remained unchanged. Headaches were decidedly less frequent, having occurred only 6 or 8 times during these 6 months, whereas they had been of daily occurrence prior to the operation. Menstruation did not return. Her weight remained unchanged. Laboratory studies at this time showed a return to normal of the glucose tolerance curve (95-122-117-91-88); blood cholesterol was reduced to 136 mg. %; and the B.M.R. dropped to −24%.

It was felt at this time that we had waited sufficiently long for any changes which might be attributed to the operation, so an attempt was now made to restore her menstrual periods. She was given a course of x-ray treatments consisting of 110 r to the pituitary region and each of the ovaries once a week for 3 weeks (so-called Mazer irradiation for secondary amenorrhea).

Before the third treatment had been given the patient had a normal menstrual period consisting of a moderate flow of 4 days' duration. This was followed by a second and a third menstrual period, each occurring after a normal interval and of normal amount.

One month after the third menstrual period the patient returned, complaining of distress in the right lower quadrant. Examination revealed a large tumor mass of indefinite outline which extended from the pelvic region well up the abdomen to about the level of the umbilicus. We suspected an arrhenoblastoma which must have grown rapidly and which had not been palpable formerly because of the obesity.

An exploratory laparotomy was performed; the mass proved to be an extensive malignancy apparently arising from the pelvis and involving the whole peritoneal cavity to such an extent that removal was impossible. A small section was removed for diagnosis and the abdomen closed. The pathologist reported a papillary adenocarcinoma of the ovary.

The patient was then given massive doses of x-ray to the entire abdomen in an attempt to control the condition. In spite of this she pursued a gradual down-hill course, developed ascites requiring frequent tapping, and died one year later.

An autopsy, performed 6 hours postmortem, revealed the following significant findings. Grossly, the abdomen was hard to enter, due to the many adhesions. The upper abdomen contained about 2500 cc. of clear, deep yellow fluid. The entire peritoneum, parietal and visceral, all the mesenteries and omenta were markedly thickened and infiltrated by yellowish firm gritty tumor tissue which bound up all the viscera and made it almost impossible to isolate each organ. It formed a heavy peritoneal sheet which hid the liver, spleen, stomach, posterior parietal wall, and coated

the bowel, kinking it quite sharply in several places. The pelvis was 'frozen' solid with the cul de sac entirely filled by tumor which extended up over the brim and was attached to the under side of the scar.

In the pituitary region there was a large cyst superior to the anterior lobe and bounded on one side by the stalk. This cyst was partially lined by a single layer of cuboidal epithelium. Some colloid-like materials were present. The anterior lobe had the usual alveolar arrangement and some of the alveoli contained a small amount of colloid. There was an abnormally large number of basophil cells, although they were outnumbered by both eosinophils and chromophobes. In one place, at the periphery of the gland, there was a small isolated collection of basophils not encapsulated, but well delineated from the rest of the gland. The posterior lobe was normal. Careful search failed to show any basophils which showed hyaline changes.

The adrenals were normal in size and gross characteristics. They were normally situated. The cortex showed a slight cellular atrophy and fatty degeneration. The medulla was normal. There were a few loose tumor cells in the surrounding tissues.

The breasts were very atrophic and pendulous. The parenchyma consisted of widely separated elongated ducts which were smoothly lined and very inactive.

The endometrium of the uterus was extremely inactive, having almost a senile appearance, with an occasional endometrial gland, but with a considerable number of stroma cells. The myometrium was normal except for invasion of tumor masses.

The tumor consisted of small groups of cells which had in many instances a central core of necrotic tissue and an outer layer of epithelium at first mistaken as being papillary. The epithelium cells were quite small, having an eosinophilic ground-glass cytoplasm and a small round or oval lightly stained nucleus with a rather coarse chromatin net. A small nucleolus was seen occasionally. Mitosis, tumor giant cells, and hyperchromatic nuclei were common. The tumor was accompanied by considerable fibrous tissue. Within the small groups of tumor were round laminated basophilic bodies.

Another section of tumor tissue had a definite tubular structure. Under low magnification this appeared to have a papillary structure, but under higher power this was disproved. These tubules were arranged in definite cords. Beyond that they cannot be said to have resembled seminiferous tubules.

DISCUSSION

The chief pathologist, who supervised the autopsy and reviewed the sections, diagnosed the case as one of tumor of the ovaries associated with definite masculinization. As pointed out by Meyer and Novak, these virilizing tumors of the ovary have a pattern which varies from a definite glandular type to a sarcomatous one. Norris (4) goes one step further by saying that, since the microscopic picture may vary so greatly, there is no real point in classifying them, and moreover, one cannot therefore make a definite diagnosis from the microscopic picture without considering the clinical data. The diagnosis of arrhenoblastoma is necessarily a clinico-pathological one. Here we have a case of virilism and amenorrhea, plus a widely metastasized ovarian tumor which has, in areas, a definite tubular pattern. The diagnosis of arrhenoblastoma, therefore, is justified.

Arrhenoblastoma, adrenal cortical syndrome and Cushing's disease had been considered carefully in the original differential diagnosis. Evidently the

marked obesity prevented recognition of the ovarian tumor by palpation in the earlier stage of its development.

It is curious that the removal of a tiny fragment of atrophic pituitary tissue, or the operative maneuvers incident to its removal should have affected strikingly the facial hypertrichosis, altered the glucose tolerance and definitely relieved the headaches. One might speculate that at the pituitary operation the surgeon punctured the pituitary cyst and obtained a specimen from within the cyst. Perhaps the decreased pressure resulting from opening of the cyst was the factor responsible for the relief of the headaches, the return of the glucose tolerance to normal, and the striking decrease in the facial hypertrichosis.

Inasmuch as the differential diagnosis between Cushing's disease, adrenal cortical syndrome, arrhenoblastoma and thymic tumor producing virilism is extremely difficult, and indeed impossible if one cannot palpate an ovarian mass or visualize an adrenal tumor, one wonders whether a simple exploratory laparotomy should not be resorted to more frequently in advanced cases of masculinization. The pelvic organs can thus be inspected and if found normal, the adrenal regions may be palpated—a much simpler and less risky procedure than exploring both adrenals surgically. Of course in the presence of a normal-sized or small sella turcica, an hypophyseal operation would never have been considered in this case.

SUMMARY

A case of arrhenoblastoma is reported. Clinical and laboratory data which 'masked' the true diagnosis are enumerated and discussed. The final diagnosis was made ante-mortem and confirmed at autopsy. No hyaline changes were found in the basophils of the pituitary.

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EDITORIAL REVIEW ARTICLE

THE CLINICAL EVALUATION OF OVARIAN RESPONSES TO GONADOTROPIC THERAPY

E. C. HAMBLEN, M.D.

OUR PRESENT concepts of endocrinopathic gynecology delineate the need for therapeutic agents possessing an ability to alter qualitatively and quantitatively abnormal ovarian functions. During the past decade there has been an increasing use of gonadotropic extracts in the treatment of various, presumably functional, aberrations of the endocrine system. For the most part these extracts have been prepared from pregnancy urine or placentae. These have been used, in most instances, for their supposed *luteinizing effects*.

The last few years, however, have seen other sources explored in the quest for more effective gonadotropes; these have included the pituitaries of animals, the serum of pregnant mares and the urine of castrated and of postmenopausal women. These gonadotropes apparently possess more ability to induce *follicle stimulation* than those of the first group.

Many conflicting reports dealing with the results of therapy with these various gonadotropes have appeared in the literature of recent years. By far the majority of these have relied upon improvement of subjective symptomatology, including alterations in amount and frequency of uterine bleeding, as proof of specific responses. This is well illustrated by the varying opinions which one encounters concerning the effects of pregnancy-urine gonadotropes upon the clinical course of meno-metrorrhagia.

Novak and Hurd (1) reported in 1931 that excessive bleeding was checked in 44 of 51 women by this form of treatment, and "often with astonishing rapidity." Geist (2), however, in a discussion of this paper at the meeting of the American Gynecologic Society, reported 24 women treated similarly without any "striking therapeutic effect." Campbell and Collip (3), the year before, had observed beneficial responses in similar patients following injections of placental extracts containing gonadotropic fractions. Nine years later, these differences of opinion still exist. Keene and Payne (4) found this method of treatment to be effective in 75 per cent of their young patients. Israel (5) has estimated that 65 to 80 per cent of women under 40 years of age "are restored to menstrual normality by intramuscular injections of 200 R.U. of the luteinizing principle, given daily during the bleeding phase and semi-weekly for 6 to 8 weeks after the bleeding has

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ceased." Wilson and Kurzrok (6) have stated that "practically every case of functional uterine bleeding can be controlled by pregnancy-urine extract, providing the dosage is adequate." They stated the average daily dose during the stage of active bleeding to be from 200 to 500 R.U. The author has failed uniformly to obtain any significant results from this form of therapy when bleeding was associated with estrogenic endometria. He (7) reported recently a consecutive series of 37 women, all of whom were hospitalized and treated with pregnancy-urine gonadotropes in daily doses as large as 8000 R.U. Only 2 of these patients showed any decrease in flow. All 37 required curettage to secure remissions from their uterine hemorrhage.

An explanation of these varied experiences is difficult. The character and severity of the irregularities vary as do their hormonal correlates. Many minor ones right themselves spontaneously. The patients in our series were admittedly ones who had failed to get relief elsewhere; many had had similar treatment previously, some with apparent temporary benefit. Variations in the actual potencies of the extracts used by different workers may have been responsible for some of the different results. Many of the extracts deteriorate rapidly after manufacture. The recent studies of D'Amour and D'Amour (8) emphasize this. Deterioration, however, cannot explain the poor results obtained by the author, for he has always made it a practice to reassay all extracts used.

Having cited these examples of divergent clinical experiences, the author hastens to observe that alterations in the subjective symptomatology of patients, including improvements in the character and cyclicity of uterine bleeding, are not per se objective proof of specific organotherapeutic accomplishments, regardless of how welcome and all-important these responses may be to the patient and her physician. The scientific evaluation of endocrine therapy must rest upon surer ground. It seems pertinent, therefore, to a better understanding of the entire problem of gonadotropic therapy to consider the various factual data which may permit a clinical evaluation of alterations in ovarian functions produced by therapy.

CLINICAL METHODS

There are certain objective observations which may supply, if interpreted with critical care, important evidence regarding changes in the functional levels of the ovaries. Only rarely is one able to make all these observations upon a single patient. These include the following.

a) The direct inspection in situ of ovaries at laparotomy, and the gross and microscopic study of those removed from patients who had received gonadotropic extracts prior to operation.

b) The microscopic study of endometria obtained by biopsy, curettage or at hysterectomy from patients who had had similar therapy.

c) Investigations of the cytologic characteristics of the vaginal fluid and of specimens of vaginal epithelium obtained by biopsy, prior to, during and following therapy.

d) Determinations of hormonal titers of bloods and of urines of patients before, during and after treatment.

e) Objective observations upon alterations in the genital system during and after therapy.

f) Similar observations upon the accessory sexual system.

g) Roentgenologic studies of the skeletal system before, during and after treatment.

The value of these observations is determined entirely by the soundness of the antecedent diagnosis of the level of ovarian function, by the correctness of the segregation of the causes operative in producing any alterations, and by the existence of unequivocal, clear-cut changes which constitute endpoints of reaction. There are no controls in clinical studies of this sort; the fidelity of the antecedent endocrine assumptions must suffice. With these general observations in mind we may proceed to analyze the data which may be secured by these various methods.

ANALYSIS OF FACTUAL CLINICAL DATA

The Study of Ovaries

Few opportunities are presented for the study of ovaries of patients who have had intensive therapy with gonadotropes. Fewer still are the cases in which the functional status of the ovary before treatment is precisely known.

Women with hypoövarianism. The diagnosis of levels of ovarian function is most unsatisfactory. Although the classical syndrome of primary (early) hypoövarianism is quite well defined, one is not always sure that the clinical correlates of this endocrinopathy are to be associated directly with a primary inadequacy of the ovaries. The striking sequellae of ovarian failure may be the result of a primary fault of function of the pituitary, thyroid or adrenal, or the exciting causes may be strictly non-endocrine in nature, resulting from inanition, constitutional disease, etc. This syndrome, however, provides our most satisfactory clinical material for studying gonadotropic responses; all of the 7 types of observations listed above may be made.

The ovarian findings to be expected after gonadotropic treatments in patients with early hypoövarianism, characterized by delayed adolescence and menarche, include enlargement, follicular activity as evidenced by maturing follicles or by follicular cysts and ovulational phenomena, or the presence of recent or organized corpora lutea. There might well be doubt in the interpretation of enlargement or of increased follicular activity, but the finding of a recent corpus luteum should supply undeniable proof of a gonadotropic response. Few direct studies of ovaries of patients of this group have been reported. The author has failed to observe any significant ovarian alterations in 5 young women with hypoövarianism who had been treated for some time prior to operation with gonadotropes derived either from pregnancy urine or from the pituitary. No direct observations upon the ovaries of patients of this group have been reported after treatments with gonadotropes prepared from the serum of pregnant mares. Since these are

apparently primarily follicle stimulating, they would seem most likely to produce significant alterations.

The striking hypoövarianism which is one of the symptoms of pituitary cachexia (Simmonds's disease) affords opportunity to investigate specific gonadotropic responses. This disease occurs much more commonly in the female than in the male: 47 of the 70 cases collected by Calder (9) were those of women. Extracts containing pregnancy-urine gonadotropes or those of the pituitary itself have been found of value at times in therapy. Hawkinson's patient (10) responded to treatment with pregnancy-urine gonadotropes and experienced a return of menstruation, with growth and hyperplasia of the atrophied sexual structures, while Moehlig's (11) patient, about the same age, failed to respond to a similar extract and died despite various other forms of endocrine therapy. The alterations which occurred in the genital tract of Dunn's patient (12) are to be related to the estrogens given rather than to the extract of anterior pituitary. The author has encountered few case reports in which autopsy findings of patients after gonadotropic therapy are described, and none in which direct studies of ovaries were made at laparotomy. Gallavan and Steegmann (13) describe the ovaries of a patient 51 years of age, who had received an extract of anterior lobe prior to her death, as being without follicles and showing marked atrophy. Probably no effect would be expected in the ovaries of a woman of this age, due to senile alterations.

No ovarian alterations have been observed following gonadotropic therapy of patients with the physiologic hypoövarianism of immaturity and of senility. Geist (14) studied the ovaries at autopsy of two infants, who had died of tuberculous meningitis and who had received injections of pregnancy-urine gonadotropes shortly before death. Their ages were 10 and 18 months. Each had received a total of 2500 R.U. of gonadotropes, the last injections being given 12 and 16 hours, respectively, before death. He observed no evidences of follicular maturation or of luteinization in these ovaries. Likewise, no specific alterations occurred in the uterine mucosae.

Watson, Smith and Kurzrok (15) have confirmed the observations of Waldeyer (16) that the ovaries of menopausal women could not be activated by gonadotropes. They found no evidences of follicular maturation in the ovaries of four women, whose ages were 43, 47, 63, and 67 years, following preoperative treatment with gonadotropes derived from the serum of pregnant mares. The oldest patient had received a total dose of 8800 M.U.

Women with alterations of ovarian function characterized by estrogenic bleeding. Most studies dealing with the direct inspection of ovaries have been concerned with searches for recent corpora lutea, the presence of which is taken as evidence of the luteinizing effect of the extracts employed.

Two general groups of patients have been investigated: *a*), women in whom it has been assumed that ovulation and the subsequent formation of corpora lutea do not occur spontaneously, and *b*) women whose regular menses lead to the belief that these normal cyclic phenomena occur spontaneously. In the first group the finding of a recent corpus luteum is taken

to be proof of a specific gonadotropic response and the absence of one, a negative response. In the second group the occurrence of multiple recent corpora lutea, or the presence earlier or later than the midmenstrual period of a recent corpus luteum which can be distinguished by morphologic attributes from the intercurrent ones, is considered evidence of the effectiveness of gonadotropic extracts. The finding of only one corpus luteum, which is thought to be correlated by its gross and histologic characteristics to the time of the cycle at which operation was performed, is thought to be evidence of no specific response. It is obvious that these two groups of patients do not furnish as satisfactory a basis for evaluation of ovarian responses as do those who present classical hypoövarianism.

We may believe that many patients, who experience irregularities of uterine bleeding or who are presumably sterile, do not ovulate, but at present *proof from a clinical point of view that they are having 'anovulatory cycles' is next to impossible.* The correctness of this assumption conditions the interpretation of recent corpora observed after therapy. Most clinical diagnoses of 'anovulatory bleeding' have been founded upon one or more observations that a patient has bled from an estrogenic endometrium rather than from a progestational one. The establishment of this fact is not conclusive proof that ovulation has not occurred as our discussion later in regard to the endometrium and the urinary excretion of sodium pregnandiol glucuronide will indicate. Admitting, for the sake of argument, that for several cycles prior to therapy it has been established that no ovulations have occurred, there would still exist the possibility that ovulation might result spontaneously during the next cycle. Studies of electrical potentials, such as those described recently (17) might aid somewhat in establishing the existence of 'anovulatory cycles' were not the procedures at present too impractical for general clinical application. In view of these facts, negative findings are more trustworthy than positive ones, and yet these are not without error. A very early corpus luteum may be missed even on the most careful inspection of ovaries in situ. Medullary corpora are quite likely to be missed in this type of examination.

Our group has reported a total of 35 patients whose ovaries were examined directly in situ following intensive gonadotropic therapy. (A large number of these ovaries were also studied histologically.) All of these patients were judged, upon the basis of proof of several antecedent episodes of estrogenic bleeding, to have been having 'anovulatory bleeding.' The majority of these patients had been given pregnancy-urine or placental gonadotropes. In some instances daily doses as large as 8000 R.U. and total doses as high as 24,250 R.U. had been employed. A few of these patients had received gonadotropes derived from the anterior pituitary or from the serum of pregnant mares. In no instances were recent corpora lutea observed. These preponderantly negative data are accepted as practically undeniable evidence of lack of response to gonadotropic therapy. Davis and Koff (18), whose studies of the effects of a gonadotropic extract from the serum of pregnant mares upon the ovaries of regularly menstruating women will be discussed later, also failed

to observe any recent corpora lutea in a small group of similar patients following a single intravenous treatment with serum gonadotropes. In several instances we have observed what appeared to have been possibly some slight follicular stimulation in the ovaries of patients of this group treated with gonadotropes derived from the serum of pregnant mares.

Women having regular menstruation. When the ovaries of women who have been experiencing apparently normal menses are examined after therapy the data secured are even less conclusive. In these women there exists to all intents and purposes a normal balance between the gonadotropic influences of their own pituitaries and their endogenous ovarian sterols. A recent corpus luteum is to be expected if operation is done during the midmenstrual phase of the cycle. Any response which follows intensive preoperative therapy must constitute fundamentally an augmentation or alteration of the spontaneously occurring ones. The augmentation of responses of sensitive and spontaneously reactive ovaries seems to the author to be an entirely different mechanism from the initiation or completion of gonadotropic responses in patients in whom these are not occurring spontaneously. This latter functional pathology is the condition which presents therapeutic problems.

The author and his coworkers have studied the ovaries of a series of 25 regularly menstruating women, who had received prior to operation intensive gonadotropic therapy for various reasons. Details of these studies have been summarized in a recent report by Ross (19). For the most part gonadotropes from the urine of pregnancy were employed. Treatments had been given during various phases of the cycle. The largest total dose of pregnancy-urine or placental gonadotropes was 5250 R.U. Treatments were spread over intervals of 3 to 12 days. In a large number of instances, microscopic studies of the ovaries were made. In only one instance were multiple corpora lutea observed; in this we did not feel justified, even from microscopic study, in attempting a segregation of intercurrent corpora from those presumably related to therapy. In the other 24 patients, corpora lutea were observed in accordance with expectations based upon the time of operation as regarded the menstrual cycle.

Each of two of these patients had received total doses of 2000 M.U. of serum gonadotropes prior to operation. Their ovaries showed apparently increased follicular maturation represented by moderate cystic alterations and some enlargement of the ovaries. An ovary of one of these patients contained a recent corpus luteum. The ovaries of the other one, who was operated upon during the first week of the cycle, contained none.

Geist (20) made similar observations upon 50 women after total doses of pregnancy-urine gonadotropes which varied between 600 and 2200 R.U. In 33 of these he observed alterations which he related to the effects of therapy. These included: an apparent increase in cystic follicles; perifollicular and intrafollicular hemorrhage; granulosal degeneration and proliferation of the thecal lutein elements. No multiple corpora lutea were observed.

There have been a number of observations, however, which do not agree with those reported by Geist and by our group.

Zondek (21) reported in 1930, upon the basis of observations made on the ovaries of 3 women who had received injections of pregnancy-urine gonadotropes prior to

operation, that cystic alterations followed this form of treatment. He reported in 1935 (22) the finding of 4 corpora lutea in an enlarged ovary of a woman 30 years of age who had received 26,500 R.U. of pregnancy-urine gonadotropes prior to operation. The injections had been given over a period of 21 days and the last injection was 27 days before operation. He concluded from this experience, as well as from observations upon other patients, that the clinical dose of pregnancy-urine gonadotropes was 15,000 R.U.

The observations of Mandelstamm and Tschaikowsky (23) in 1932 are striking because of the number of positive findings and the smallness of the dose of pregnancy-urine gonadotropes given. Total doses varied from 400 to 1100 M.U. Ten women were studied at operation following these treatments. They observed degenerative changes in the maturing follicles. There was an increased number of follicular cysts and numerous corpora lutea were encountered in the ovaries of many of the patients. These authors concluded that these ovarian changes were sufficient to produce a temporary sterility.

Westman (24) in 1935 made observations somewhat similar to those of the last two authors. He found the ovaries of 3 patients, who had received total doses of pregnancy-urine gonadotropes ranging from 3600 to 5000 R.U., contained cystic follicles, hemorrhagic areas and recent corpora lutea. The endometria, likewise, were in the progestational phase. He concluded that the responses of the ovaries of experimental animals and those of woman respond in the same manner to this type of treatment.

Büttner (25) has reported recently the production of multiple corpora lutea in the ovaries of a small series of normally menstruating women by treatment combining pregnancy-urine gonadotropes and those from the pituitary or from the serum of pregnant mares. In 4 women, treated in this manner, he observed at laparotomy 2 or more recent corpora lutea, the occurrence of which he attributed to the therapy.

Engle (26) considered this work of Büttner's to be probably the most promising indication to date (1937) of the functional activation of ovaries by chorionic gonadotropes. He regarded the objective data based upon finding supernumerary corpora lutea to be free from criticisms which might be urged against the work of Waldeyer or Westman. The observations of Büttner are in good agreement with the dual action of these two types of gonadotropes in the monkey which was described by Engle and Hamburger (27).

Specific effects from therapy with gonadotropes derived from the anterior lobe of the pituitary have not been reported by most workers. It has been assumed generally that extracts of these had not been sufficiently concentrated to warrant the expectation of any striking ovarian alterations from their use. Our group was unable to observe any significant alterations in the ovaries of 7 patients who had had total doses of these gonadotropes which ranged from 100 to 360 R.U. prior to operation. Stöckl (28), however, reported positive findings in the ovaries of 3 patients who had received total doses of these gonadotropes ranging between 200 and 400 M.U. He reported observing an intense hyperemia of the pelvic organs and the occurrence of many hemorrhagic follicles in the ovaries. In one of the patients, who was past menopause, he found an unusually large corpus luteum. Alterations in the endometria were also reported.

There has been a more general agreement among various workers regard-

ing the ovarian responses to serum gonadotropes. The experience of our group has been referred to. Kurzrok (29) reported that, when adequate quantities (3600 M.U.) of these gonadotropes were given during the early phase of the menstrual cycle to women in the third decade of life, a marked increase in the size and weight of both ovaries occurred. These ovaries contained many large follicular cysts. He reported, however, that the ovaries of women in the fifth decade did not react to this form of treatment, even when 9000 M.U. of the principles were given. These studies were reported subsequently in detail by Watson, Smith and Kurzrok (15). Moricard and Saulnier (30), Siegmund (31) and Westman (32) made similar observations regarding follicle stimulation induced by these gonadotropes.

None of these previous studies, however, had indicated that ovulation and the formation of corpora lutea occurred as a result of these treatments. Davis and Koff (18) have described findings of this nature. In half of a group of 26 women, who for the most part were considered to have normal menstrual cycles, recent corpora lutea were observed in the ovaries at laparotomy. As a rule a single intravenous dose of serum gonadotropes had been given several days prior to operation. In approximately 33 per cent of these patients the ovaries contained multiple corpora lutea. These findings were interpreted by the authors as indicating that multiple ovulations had been produced by therapy.

Our group has made no direct observations upon ovaries after intravenous administrations of these serum gonadotropes. We have, however, made pertinent observations after this form of therapy (discussed below under *endometrial studies* and *hormonal titers*) which lead us to believe that treatment, as given by Davis and Koff, will not yield complete physiologic ovarian responses in patients in whom failure of function exists.

Recent studies by Hartman (33) upon the effects of serum gonadotropes on the ovaries of adult monkeys warrant consideration at this point. These are based upon 104 experiments upon monkeys of apparently low fertility and presumably not spontaneously ovulating. The majority of the observations followed intravenous injections of the same preparation of serum gonadotropes used by Davis and Koff in their studies. Similar doses were employed. In 32 instances the animals were entirely refractive to therapy. Overstimulation of the granulosal elements occurred in 16 cases. In these cases ovulation could not be produced by additional therapy with pregnancy-urine gonadotropes. There occurred only "7 unequivocal cases in which ovulation may rightly be attributed to the injections."

Hartman called attention to the marked damage to the follicular apparatus which was observed in a large number of instances. He observed that "from the protocols of this series of experiments it is possible to extract a recipe for the best method of injuring the follicular apparatus of the monkey." The employment of strong luteinizing principles following overstimulation of the ovaries with serum gonadotropes was shown to cause 'a violent disruption of the granulosa.' He observed however, that these deleterious effects were temporary and that recovery was prompt and complete, and he asserted that these findings would not lead one to "condemn the use of gonadotropic extracts in attempts to help sterile women when they are desperately desirous of having children."

Hartman regarded the failures reported by him to be related in a large degree to the difficulty of estimating a correct dosage for the individual. He called attention to the fact that two adult healthy monkeys may respond quite differently: in one the intravenous injection of 30 R.U. of serum gonadotropes might have no effect, while in the other the ovaries might be disrupted temporarily. Similar clinical difficulties doubtlessly exist. When these principles are used the patient should be followed closely in the manner described later in the discussion of therapeutic tests.

By way of recapitulation at this point of the discussion, it is evident from these observations that no definite proof has been brought forward that gonadotropes are capable of calling forth complete ovarian responses in patients whose ovaries are not responding spontaneously to the endogenous pituitary gonadotropes. Augmentation or alteration of the normal ovarian-pituitary system should be differentiated distinctly from a mechanism whereby truly hypofunctioning ovaries are caused to undergo complete physiologic responses.

Endometrial Studies

The study of repeated biopsies of endometria may permit an evaluation of endometriotropic influences. Fluctuations in the biphasic endometriotropic influences may be correlated with similar biphasic gonadotropic ones. Observations which have aimed at gauging functional ovarian levels by studies of endometrial architecture assume that the endometrium maintains at all times a uniform sensitivity to the ovarian sterols with the result that alterations in the function of the ovaries are reflected promptly and characteristically in the histology of the endometrium. This assumption is not true. We have reported objective evidence that varying grades of endometrial nonreceptivity or refractivity may exist. We (34) have observed patients who excreted normal amounts of sodium pregnandiol glucuronide (metabolic product of progesterone) although their endometria showed no progestational changes. In other patients who were receiving cyclic therapy with estrogens and progesterone we have observed (35) some evidence that endometria may be refractive or certainly not normally sensitive to both of these sterols. As a result of these studies we do not feel that the endometrium can be used as a strictly reliable diagnostic mirror to reflect the level of ovarian function. Ross (36) has drawn similar conclusions.

In general, two types of endometrial changes may be observed during the course of therapy of patients upon whom repeated biopsies have indicated the spontaneous occurrence of no recognizable cyclic alterations, or of only minimal changes progressing no further than to result in some degree of estrogenic proliferation. One of these concerns the alteration of a hypoestrogenic (atrophic) endometrium into one indicative of a more normal degree of estrogenic activity. When these alterations follow gonadotropic therapy, it has seemed feasible to explain them upon the basis of a stimulation of the follicular apparatus resulting in the outpouring of more estrogens. The other type of endometrial change concerns the alteration of various estrogenic

endometria into ones which show well differentiated progestational characters. This effect has been explained as being due to a luteinizing action of the gonadotropes resulting in the formation and secretory activity of the lutein apparatus and in an increased elaboration of progesterone.

The end-points in these endometrial alterations are not easy to read in many instances despite the readiness with which even the unskilled microscopist can differentiate an atrophic from a progestational endometrium. The questions to be answered in these studies are whether there has occurred an increased elaboration of estrogens or a continuation of estrogenic activity and, in addition, formation, metabolism and utilization of progesterone. When one encounters patients who bleed cyclicly from estrogenic endometria despite the urinary excretion of normal titers of sodium pregnandiol glucuronide and when one observes other patients who continue to bleed from endometria of estrogenic characters despite the cyclic administration of progesterone, how can one say with any degree of assurance that these endometria, estrogenic though they may appear, have not been subjected to the action of progesterone?

Our group has made numerous endometrial studies after gonadotropic therapy of patients who were presumed to be experiencing 'anovulatory' bleeding due to the repeated findings of estrogenic endometria by biopsy at the onset of their flowings. In not one instance, despite many series of therapy with observations extending over periods as long as 8 to 12 months and despite the employment of all of the various gonadotropes in different dosages have we encountered a single alteration of an estrogenic endometrium into one of recognizable progestational character.

For example, one series of 37 consecutive patients of this type who were treated with pregnancy-urine or placental gonadotropes was reported in which not only were there no histologic alterations in the endometria, but also no changes in the character of bleeding except in 2 instances.

It has been observed, however, in a small group of patients in whom bleeding occurred from mixed endometria (those with poorly differentiated progestational characteristics, which often showed patchy distribution) that treatments during the latter part of their cycle with pregnancy-urine or placental gonadotropes often resulted in more normal bleeding from endometria exhibiting more complete progestational differentiation.

Our group has studied recently a small series of 5 patients being treated because of ovarian sterility. All of these patients had cyclic bleeding, although endometrial specimens obtained repeatedly at the onset of their flowings had shown no progestational differentiation, and determinations of their urinary titers of sodium pregnandiol glucuronide indicated these to be markedly decreased in most instances. Repeated cyclic treatments with serum gonadotropes given intramuscularly daily for 7 to 8 days during the early interval phase and concluded on the 12th to 14th day of the cycle with an intravenous dose, similar to that given by Davis and Koff (18), failed to result in any alterations in the endometrial architecture or in the urinary titers of the pregnandiol complex.

In summary, no evidence has been secured from studies of endometrial specimens taken after therapy, that gonadotropic extracts are able to alter the ovarian-endometrial disturbance of patients who bleed from estrogenic endometria. The negative findings from both the direct inspection of ovaries and from histologic examination of the endometrium may permit two opposing interpretations: *a*), that the various gonadotropes lack, in the human female, the specificity which they exhibit in experimental animals; or *b*), that lowered receptivities of ovaries and of endometria in patients bleeding from estrogenic endometria prevent specific actions which might have occurred under more favorable circumstances.

The author has submitted recently (35) evidence for the belief that, perhaps, ovarian and endometrial refractivities should be considered factors in the etiology of estrogenic bleeding. If this assumption be correct, we have chosen an unfair group of patients for the clinical evaluation of gonadotropes. The findings of Büttner (25) and of Davis and Koff (18) suggest that gonadotropes may be specific when normal ovarian receptivity exists.

Vaginal Cytology

Papanicolaou (37) has found that striking alterations occur in the cytology of the vaginal fluids of normal women during the menstrual cycle and during pregnancy. The recognition of these various fluctuations during the menstrual cycle of normal women is far from easy except in ideal patients. Alterations in the vaginal cytology of menopausal patients after treatment with estrogens have been reported by Salmon and Frank (38).

It would seem that patients with early hypoövarianism resulting in delayed adolescence would be ideal for such studies. An alteration in the cytology indicative of more adequate estrogenic metabolism could be taken as evidence of the follicle-stimulating effect of gonadotropic therapy. Our experience in a relatively small series of patients of this type has not permitted any clear-cut deductions.

The histologic investigation of vaginal biopsies has a limited usefulness in supplying data regarding ovarian responses. This method of approach, however, would seem to be of value in those patients who show an atrophic vaginal epithelium. Of this group, climacteric women should be excluded since ovarian refractivity due to senility would extend little hope of effective gonadotropic therapy. Juvenile females are not treated commonly with gonadotropes for obvious reasons and, furthermore, vaginal biopsies are usually out of the question; therefore, this group of patients is not investigated as a rule. Patients with primary hypoövarianism resulting in delayed adolescence may be studied at times by this method. These, however, despite striking evidence elsewhere of ovarian deficiency, may show at times fairly well proliferated vaginal epithelia. The alteration by gonadotropic therapy of an atrophic vaginal epithelium into one showing histologic characteristics of sexual maturity should be good clinical evidence of effective follicle-stimulating influences resulting in an increased estrogenic metabolism. No significant studies of this character have been reported.

Hormonal Titers

Theoretically studies of the titers of the various sex sterols and of the gonadotropes in the blood and urine of patients before, during and after gonadotropic therapy should yield significant factual data. If a hypothetical patient has low titers of estrogens and negative values for progesterone (or its metabolic products) and if the titers for estrogens increase and progesterone or its metabolic products appear in the urine and blood after gonadotropic therapy, this should be excellent proof of an induced biphasic ovarian response. Unfortunately, these theoretical assumptions are difficult of realization due to technical as well as clinical limitations.

There seems to be an almost universal belief at present that no significant quantitation of the sex hormones in the blood is possible. There are no methods which employ small amounts of blood (those at present require up to 40 cc. for a single determination) which permit frequent serial determinations which seem necessary for the recognition of the many transient fluctuations which must occur in the cyclic endocrinokinetic system.

The studies of urinary titers, while more satisfactory than those of the blood, leave much to be desired. In general, there are two major difficulties in regard to the authenticity of the data obtained; these difficulties apply particularly to the sex sterols and, perhaps, in a restricted or limited way to the gonadotropes.

The first of these concerns the fact that sterols, in particular, are excreted in the urine in conjugated and often altered chemical forms which may possess lowered or absent pharmacologic activity. These chemical alterations include esterification with glucuronic and perhaps other acids. Hydrolysis of these esters is a necessary preliminary step to quantitation either by colorimetric methods or those of bioassay. There frequently exists uncertainty as to completeness of the hydrolysis and as to possible destruction of the active principles from deterioration before extraction or during the process of hydrolysis with strong acids.

The other difficulty concerns the fact that various factors influence the amount of sterol which is excreted. In the metabolism of progesterone we must consider the following factors: *a*), ovarian, involving the formation and secretion of progesterone; *b*), endometrial, concerned apparently with the activation and utilization of progesterone and its alteration into inactive pregnandiol; *c*), hepatic, responsible for the conjugation of pregnandiol with glucuronic acid; and *d*), renal, which is concerned with the excretion of sodium pregnandiol glucuronide. Disturbances of any one of these four systems will result in alterations in the urinary titers of sodium pregnandiol glucuronide. Similar complexities doubtlessly occur in the metabolism of estrogens and androgens. The recent studies of Smith and Smith (39) have indicated the complexities of estrogenic metabolism.

Technical difficulties in the methods of quantitation render more uncertain the data secured from these sources. These errors are multiplied, in most instances, when the smaller amounts of hormones associated with the

menstrual cycle are investigated although the methods yield figures that are none too reliable when the hormonic plethora of pregnancy is studied. At present, for the problem under investigation, methods of bioassay for the determination of urinary gonadotropes, estrogens and androgens, as well as the colorimetric methods for estrogens are unsatisfactory. The gravimetric method for the determination of sodium pregnandiol glucuronide, described by Venning and Browne (40), despite its time-consuming features and despite, as we have noted, difficulties of segregating individual variants, has alone permitted satisfactory application to our problems.

Studies by our group of the urinary titers of the pregnandiol complex before, during and after gonadotropic therapy have yielded, up until the present time, little evidence that the formation and metabolism of progesterone have been altered. For the most part these studies have been concerned with patients having estrogenic bleeding associated with low or negative urinary values of pregnandiol complex. As we have noted already, in a small group of patients bleeding from mixed endometria, therapy with pregnancy-urine and placental gonadotropes has apparently resulted in more normal bleeding, in a more complete progestational proliferation of the endometrium and in an increase in the urinary excretion of the pregnandiol complex.

So far, our studies of the urinary excretion of the pregnandiol complex during and after gonadotropic therapy of patients who presented delayed adolescence and other typical symptoms and signs of early hypoövarianism have yielded no significant data. Suitable tests for estrogens would be of value in these instances in judging stimulation of the follicular system, which obviously must occur before a suitable structural substrate is prepared for progestational phenomena. In certain of these patients, in whom the hypoövarianism is associated with disgerminal characteristics and potentially androgenic tendencies, sensitive methods of determining androgens might show increases in these values concomitant with the progress of gonadotropic treatment.

Browne and Venning (41) have reported important observations upon the effects of pregnancy-urine gonadotropes in 7 patients with more or less cyclic bleeding associated with low urinary values of sodium pregnandiol glucuronide and functional sterility (the endometrial status of these patients was not investigated). They found when injections of 100 M.U. daily were started after the beginning of excretion of the pregnandiol complex, that bleeding was postponed for as much as 17 days and that there was an increase in the amount of and duration of excretion of sodium pregnandiol glucuronide. Such an action is in keeping with one of the presumed rôles of these gonadotrpoes in pregnancy: the prolongation of activity of the corpus luteum until such time that its function is taken over by the chorio-placental system. These observations agree with the author's experience with certain patients with cyclic or acyclic bleeding associated with mixed endometria and low urinary titers of sodium pregnandiol glucuronide.

Alteration in the Genital System

Significant clinical observations regarding alterations in the genital system may be made during the course of gonadotropic therapy. These are concerned with the growth and sexual maturation of the hypoplastic genitals of patients with early hypoövarianism. These alterations are to be related to an increased elaboration of estrogens as the result of follicle-stimulating effects of the injected gonadotropes. The value of these observations is conditioned by the care and meticulousness of the clinician. Mensuration, graphic drawings, and photography often make more certain the mental impressions of growth processes. The processes of growth may be observed in the external genitals; in the depth, caliber and degree of differentiation of the vagina; in the cervix, where particular significance is attached to its size and the ratio between its length and that of the entire uterine body (cervico-uterine ratio); in the uterus, where careful hysterometric data are of value as well as observations dealing with alterations in the contour and in the cervico-uterine ratio; and in the ovaries, where careful bimanual examinations may yield significant information regarding growth and cystic alterations.

Turner (42) has reported that gonadotropic extracts of anterior pituitary gland were effective in producing signs of sexual maturation in 2 patients who presented a syndrome characterized by infantilism, congenital webbed neck and cubitus valgus (Turner's Syndrome). One patient, aged 16 years and 3 months, who had failed to respond to growth-promoting extracts of the anterior pituitary, showed the following clinical responses after receiving pituitary gonadotropes in doses of 62.5 R.U. 3 times weekly for 30 days: beginning growth of pubic hair; an increase in the lumen of the vagina with an increase in depth from 1.6 to 3.5 inches; and an enlargement of the uterus (not palpable before treatment) to a depth of 2.2 inches. The subsequent treatment of the patient, since it also involved estrogens, cannot be evaluated in terms of gonadotropes. It is significant, however, that subsequently two episodes of uterine bleeding followed. Ovaries were never thought to be palpable. Another patient, aged 18 years, failed to respond to growth-promoting extracts. She was given subsequently over a period of 7 months a total dosage of 4375 R.U. of a pituitary gonadotrope. The following alterations were observed: some growth in pubic hair; a slight increase in the lumen of the vagina; the uterus and ovaries remained unpalpable; and breasts showed no hyperplasia.

Payne and Shelton (43) treated 10 patients who had uteri which measured less than 2 inches in depth with a gonadotropic extract of anterior pituitary gland. In addition, 3 of these patients received either thyroid substance and/or estrogens. Most of these patients had experienced moderate irregularities of bleeding. Some enlargement of the uterus was found to occur in 3 of the 7 patients who had received only gonadotropes. These increases in depth of the uterus were as follows: from 1 1/4 to 3 1/4 inches in one case; from 1 3/8 to 3 inches in another, and from 1½ to 2 1/4 inches in another. It should be observed, however, that care must be exercised in the interpretation of data of this sort obtained from women having menstrual cycles as it is well known that cyclic alterations occur in the size and depth of the uterus.

Accessory Sexual System

Observations similar to those upon the genital system may be made upon the accessory sexual structures of patients with delayed adolescence related

to early hypoövarianism. These observations are concerned with the obvious signs of sexual maturation and feminization, and include such commonly-made ones as those dealing with growth of sexual hair, alterations in the voice, hyperplasia of the breasts, development of typical localized deposits of fat and changes in the texture of the skin. Mensuration, anthropometric measurements and photography of the nude supply a more objective character to these impressions.

The Skeletal System

Roentgenologic studies of the epiphyses of the various long bones may supplement other observations following gonadotropic therpay upon patients with delayed adolescence due to early hypoövarianism. Characteristically in these patients the epiphyses remain ununited. After successful therapy, due fundamentally perhaps, to increased estrogenic metabolism, roentgenologic evidence of progressive osseous maturation may be obtained.

PROBLEM OF A THERAPEUTIC TEST

There is apparent need at times of giving certain patients a trial of gonadotropic therapy. It has been suggested earlier in this paper that the receptivity of the end organs (ovaries) is of prime importance in conditioning the clinical response. It has been inferred also that most patients, who have estrogenic bleeding as the basis of their irregular flowing or their sterility, have ovaries which lack normal sensitivities to endogenous gonadotropes. The results of clinical observations to date seem to indicate clearly the ineffectiveness, and perhaps the undesirability, of gonadotropic therapy of these patients. There is at present, however, no clear-cut way of judging the responsiveness of ovaries except by way of an adequate trial of gonadotropic therapy. This seems to be desirable particularly in patients with delayed adolescence and the other characteristic symptoms and signs of early hypoövarianism.

In embarking upon a therapeutic test, the first question which arises is that of what gonadotropes to use. Since the primary desideratum in these patients with genital hypoplasia is to produce growth and sexual maturity of the ovaries (and in turn, thereby, similar alterations in the genital tract), the use of those gonadotropes which are primarily follicle stimulating in character seems rational. The dosage and method of administration are next to be considered. Unfortunately, the question of dosage hinges upon clear-cut reports of positive responses, of which there have been few. The daily doses may be approximated for the various gonadotropes considered likely to prove effective, as follows: antex (serum gonadotrope) 200 mouse units; gonadogen (serum gonadotrope) 20 to 40 rat units. The injections are given intramuscularly, after preliminary allergic studies have established the existence of no untoward sensitivity. Treatments with one of these serum gonadotropes are given daily for 3 to 4 weeks, during which time careful clinical observations, as outlined and described above, should be carried out. Bimanual abdomino-pelvic or abdomino-rectal examinations should be made

twice weekly to evaluate alterations in the genital organs; attention should be directed particularly to the ovaries, and any undue cystic alteration should call for a temporary discontinuation of treatments. At the onset, uterine and cervical hypoplasia may not permit hysterometric or endometrial studies, but if enlargement occurs these should be made. Daily treatments should not be continued longer than 3 to 4 weeks due to a likelihood, unproven but theoretically possible, of provoking a secondary refractivity or lowered receptivity of the ovaries to the therapeutic agent being employed. A rest period of about 4 weeks should intervene and then another series of therapy should be given. In all, if necessary, 3 to 4 of these series should be given. When it becomes apparent during one of these series of therapy that a full estrogenic response has been obtained (i.e., when a well proliferated estrogenic endometrium is found on biopsy), an intravenous injection of a suitable serum gonadotrope (i.e., gonadogen 40 to 80 R.U.) seems advisable. This is followed by daily injections of gonadotropes, derived from pregnancy urine, in doses ranging from 500 to 2000 R.U. for 14 days. During this portion of the treatment, urinary excretion of sodium pregnandiol glucuronide may be studied and at the conclusion of this treatment, or at the onset of any bleeding that may occur, endometrial studies should be made with the hope of observing progestational alterations. If positive reactions are obtained, which indicate that a complete biphasic ovarian response has followed therapy, no further treatment should be given for at least 6 to 8 weeks, but observations should be continued, in order to test the spontaneity of these adjustments. If spontaneous ovarian responses do not follow, complete treatments using both types of gonadotropes, should be repeated.

For illustration of the application of therapeutic tests a case in which a desirable response was obtained is reported.

G. R., a single colored girl, 17½ years of age, had not begun to menstruate and had shown signs of incomplete sexual maturation. On examination she presented a classical syndrome of juvenile hypoövariansim. Her span exceeded her height by 5 inches and her lower measurement exceeded the upper one by 6.5 inches. Hands and feet were large and slender with long tapering digits. Her weight was 105 lb. despite a height of 67.5 inches. Characteristic feminine padding was absent. There had been slight physiologic hyperplasia of the breasts. There was a slight growth of pubic hair. The external genitals were markedly hypoplastic. The vaginal cavity was approximately 1.5 inches in depth; the fornices were undeveloped; the walls were thin, smooth and relatively inelastic; vaginal discharge was scanty. The cervix was flush with the upper vaginal vault and, in its size and character, resembled an urethral meatus more than a cervix. A small, flat, triangular uterus without convexity of the fundal portion was identified. The entire uterus and cervix were estimated not to exceed 1.5 inches in greatest dimension. A sound could not be introduced because of the hypoplasia; therefore, no endometrial biopsy was possible. Ovaries could not be palpated, nor any adnexal structures identified.

The basal metabolic rate was −4 per cent. Roentgenologic studies showed the sella turcica to be normal, and studies of the ankle, knee, wrist and elbow showed ununited epiphyses. The osseous age was estimated to be approximately 15 years.

No treatment was given except that of injections of gonadotropes. The patient

was given injections of 200 R.U. of a gonadotropic extract from the pituitary (gonadotropic factor) on Oct. 28 and 30 and on Nov. 2, 4, 6, 9, 13, and 16 the total dosage was 1600 R.U..

Clinical observations, including bimanual pelvic examination and cytologic studies of the vaginal fluid, yielded no significant data, except that there was an increase in the leukocytes of the vaginal smears on Nov. 4th and 16th.

At this point treatment with a serum gonadotrope ('antex') was begun. Doses of 100 M.U. of this gonadotrope were given on Nov. 20, 23, 35, 27, and 30 and on Dec. 2, 4, 7, 11 and 14, making a total dosage of 1000 M.U. Examination on Dec. 16th showed a definite increase in the size of the genitals and of the vagina. The cervix and uterus had increased sufficiently in size so that a regular uterine sound could be inserted. This procedure indicated a uterine depth of 2 inches. An endometrial biopsy was made and the tissue obtained showed upon microscopic examination a good estrogenic response. On bimanual pelvic examination, the left ovary was felt and judged to be somewhat cystic and about normal size.

Treatment was discontinued at this stage to avoid possibility of the development of a secondary refractivity to the therapeutic agent.

The patient reported back on Jan. 13th (after an interval of 4 weeks). There had been no menstruation. Slight regression of the effects of treatment had occurred. An additional series of treatments with antex was given as follows: doses of 200 M.U. were administered on Jan. 13, 15, 18, 20, 22, 25, 27, and 29 and Feb. 1, 3, 5, 8, 10, 12, 15, 17, and 22, the total dosage for the series being 3400 M.U. On Feb. 3, both ovaries were felt on bimanual pelvic examination and they were estimated to be about one-half normal size. Studies of vaginal cytology showed nothing significant except a disappearance of leukocytes on Feb. 10 and 12. At the conclusion of the series of therapy on Feb. 24, sounding of the uterus showed it to be 2.5 inches in depth. An endometrial biopsy was made and examination of the tissue obtained showed a well developed progestational endometrium. Bimanual examination indicated the left ovary to be about normal in size and to have a soft cystic feel. The right ovary remained small. Treatment was discontinued at this point to await developments and to test the therapeutic value of the alterations produced.

On Feb. 26, the patient began her first menstrual period which continued until March 2, and which appeared normal in all respects. The patient returned for further observation on March 24. She had had no therapy since Feb. 22. She had had no further menstruation. Genital structures had shown no regressions since last examined. The uterus measured 2.75 inches in depth. An endometrial biopsy was made; the tissue obtained showed a normal estrogenic endometrium. No treatment was initiated; observations were continued.

On April 7, another biopsy of the endometrium was made. The endometrium at this time showed a well differentiated progestational response. On April 8, the patient's second menstrual period began. It continued until April 11 and was normal in all respects. No further treatments were advised. Her last visit was on Aug. 18 when she reported regular menses each month since April. Her last period had begun Aug. 4 and had lasted 6 days. A general survey showed the following evidences of sexual maturation: and increase of weight to 115 lb. with fat localized in a typical feminine fashion; increased hypertrophy of breasts; increase in pelvic and axillary crines; continued hyperplasia of the genital structures; roentgenologic evidence that all epiphyses except those of the radius and ulna had closed; and ovaries, uterus, cervix and vagina appeared normal in size and character.

SUMMARY

This review has pointed out the various difficulties inherent in attempts to evaluate clinically ovarian responses to gonadotropic therapy. Pertinent methods of clinical observation have been described. The need of therapeutic tests for the gonadotropes has been considered and the advisability of such to determine the receptivity of the ovaries has been pointed out. A method for carrying out what would seem to be a satisfactory therapeutic test has been described. Consideration of the clinical data available indicates that there exists little definite proof that therapeutic efficiency has been established for the gonadotropes in most supposed endocrinopathic gynecology. Augmentation of normally occurring ovarian responses may be produced by injections of gonadotropes. The existence of varying degrees of ovarian and endometrial refractivity or lowered receptivity may be responsible for failure of desired therapeutic results in functional bleeding and in ovarian sterility, rather than lack of specificity of the gonadotropic extracts. Patients with symptoms and signs of primary or early hypoövarianism seem most likely to be benefited by this therapy. These should be given an adequate trial of gonadotropic treatment before replacement therapy with the ovarian sterole is initiated.

The following commercial preparations of gonadotropes have been supplied by the manufacturers for our studies. Those derived from pregnancy urine or placentae: antuitrin-S (Parke, Davis & Co.), follutein (E. R. Squibb & Sons), A.P.L. and physex Leo (Ayerst, McKenna & Harrison). Those derived from the pituitary: antuitrin, gonadotropic (Parke, Davis & Co.), gonadotropic factor (Ayerst, McKenna & Harrison). Those derived from the serum of pregnant mares: antex Leo (Ayerst, McKenna & Harrison), gonadogen (Upjohn Co.). Those derived from urine of menopausal and castrated patients: gamone (E. R. Squibb & Sons).

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CLINICAL AND LABORATORY NOTES

COMPARATIVE POTENCY OF COMMERCIAL ANTERIOR PITUITARY-LIKE PREPARATIONS*

FERDINAND GAENSBAUER AND JAMES T. BRADBURY†

A UNIFORM method of standardization for anterior pituitary-like preparations derived from pregnancy urine has not yet been adopted by pharmaceutical manufacturers. Consequently, the biological unity of potency, the rat unit, varies from product to product. These extracts have obtained widespread use in the treatment of cryptorchidism and menstrual disturbances. Thompson, Heckel, and Bevan[1] used two of these pregnancy urine derivatives, follutein and APL, in the treatment of undescended tests, but were unable to correlate dosage because quantitative comparisons between the two were not available. In the present study a direct comparison of the potency of the better known anterior pituitary-like products has been made.

Materials. Five commercial products, antuitrin-S, APL, antophysin, follutein, and pregnyl, which were purchased through regular drug dispensaries, were compared. Antophysin and pregnyl are sold as dry powders claimed to be indefinitely stable until made up into solution with distilled water, after which they gradually deteriorate. The diluent of antophysin contains 0.2% metacresol as a preservative, and that of pregnyl 0.3% tricresol. Antuitrin-S and APL are aqueous solutions with 0.5% phenol as a preservative. Their labeled potency is guaranteed for 6 and 8 months after manufacture respectively if they are stored in a refrigerator. Antuitrin-S is marked with the date of manufacture; the date of expiration appears on the APL package. Follutein is marketed as a glycerin concentrate with date of expiration a year after manufacture; but after the diluent of distilled water has been added, the labeled potency is maintained for only 2 months. APL, antuitrin-S, and follutein were all tested well before the expiration date. Only the 10 cc. vials of a strength of 100 R.U. per cc. were utilized. A similar procedure was not possible with pregnyl since it is distributed solely in 100 R.U. ampules.

The details of the commercial standardizations of the different preparations as obtained from the descriptive literature accompanying each package are contrasted in table 1. A vaginal estrous end point has been assumed for antophysin, since the advertising literature is very indefinite. Despite variations in technique of injection and duration of the test period it is apparent that one of two end points has been adopted, either vaginal estrus or luteinization of the graafian follicle.

Methods. The method of assay selected for this study was essentially that described by Rowe, Simond, and Nelson.[2] Immature female albino rats, 26 days old, weighing from 35 to 50 gm., were employed.‡ Usually 10 rats were used on each

* This study was made possible by a grant to the Department of Obstetrics and Gynecology from the Faculty Research Fund.

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‡ The rats were obtained from an inbred strain of laboratory stock raised by Woodruff and Son, Berkley, Michigan.

dose level, and were injected subcutaneously twice daily for 3 days. The commercial extracts were diluted, so that each injection was 0.25 cc. and the total amount injected was 1.5 cc. The dilutions were made by adding 0.2 cc. of products labeled '100 rat units per cubic centimeter' to 30 cc. of distilled water for testing at a 1 R.U. level; 0.4 cc. to 30 cc. for the 2 R.U. level; and 0.4 cc. to 20 cc. for the assay at the 3 R.U. level. The animals were killed 96 to 100 hours after the first injection,

TABLE 1. SUMMARY OF COMMERCIAL ASSAY METHODS

Preparation	Age of rats days	Wt. of rats gm.	No. of injections	Duration of test hours	End point	Unit amount	Comparative potency
Antuitrin-S	26–28	—	6	96–100	Corpora lutea	Total	1.0
Fullutein	30	—	6	100	Corpora lutea	Total	1.0
Pregnyl	21–28	25–35	6	92	Vaginal Estrus	Total	0.5
Antophysin	26–28	45–60	6	120	[1]Vaginal Estrus	Total	0.5
APL	21	28–40	3	120	Vaginal Estrus	Daily Dose	0.5

[1] Descriptive literature indefinite.

that is, at 30 days of age, and the ovaries and the uterus were removed in toto; the uterus was severed from the vagina at the cervico-vaginal junction. To determine more accurately the degree of ovarian change the ovaries were then freed from the periovarial capsule and examined under a binocular microscope at 10 diameters magnification. Uterine weights were obtained after the fallopian tubes had been removed and the fluid expelled by pressing the uterus between layers of filter paper. Other pertinent data were recorded, such as vaginal patency and smear, ovarian weights, and gross descriptions of the uteri and ovaries.

Results. All products were assayed at a labeled potency of 1 R.U., and the results are listed in table 2. At this dose only antuitrin-S and follutein gave vaginal estrous and luteinization responses in a majority of the test rats.

Antophysin, APL, and pregnyl did not give a response in the majority of the rats at the 1 R.U. level, so they were reassayed at 2 or 3 R.U. levels. The results are given in table 3. The percentage of positive vaginal estrous and luteinization responses at the 2 R.U. level compares favorably with the 1 R.U. assays of antuitrin-S and follutein in table 2.

Additional assays, not listed, on 2 other strains of albino rats have confirmed the findings tabulated above. The results indicate that the 3 preparations, antophysin, APL, and pregnyl (see table 3), are actually about half as potent as antuitrin-S and follutein, though all are advertised as '100 rat units per cubic centimeter.'

Discussion. Anterior pituitary-like extracts administered to immature female rats produce morphological changes in the gonads, consisting mainly of follicular growth and corpus luteum formation. As a result, sex hormones are elaborated which, in turn, cause the reproductive tract to mature. Methods of determining the potency of anterior pituitary-like preparations are dependent on the morphological changes in the gonads or on the degree of development of the sex accessories, such as the vagina or the uterus.

A method of bioassay based on the formation of corpora lutea in the ovary has been employed by two manufacturers. The corpus luteum end point is not clear-cut, since an examination of ovaries under 10 diameters magnification shows stages of transition from hyperemic follicles through ruptured follicles to more advanced stages of luteinization. In an assay using 10 rats, each receiving 2 R.U. of antophysin, there were 10 hyperemic follicles, 14 ruptured follicles, 8 early corpora lutea, and no well-developed corpora lutea, stages which were graded as reactions I, II, III, and IV,

respectively. In the present study reactions II, III, and IV were recorded as corpora lutea. This end point in commercial bioassay is less subject to such a wide range of personal interpretation, since the ovaries are examined in the animal with the periovarial capsule still present, and, consequently, only the more distinct corpora lutea are visible.

The other end point used in commercial assay, that of vaginal estrus, is determined simply by examining a vaginal smear for the presence of cornified epithelial cells. D'Amour and D'Amour demonstrated that the amount of pooled pregnancy urine necessary to induce vaginal estrus is 0.36 cc. and that the amount necessary to produce luteinization of the graafian follicle is 0.48 cc. Thus the potency of the vaginal estrous unit tends to be less than the unit standardized by the corpora lutea end point.

An increase in the ovarian weights of treated rats over those of controls has been utilized as an end point in the assay of gonadotropic extracts of the pituitary[3] and pregnant mare's serum.[4] In doses just sufficient to bring about vaginal estrus or luteinization of the graafian follicle anterior pituitary-like extracts, in contrast to gonadotropic pituitary extracts and pregnant mare's serum, produce no significant increase in ovarian weights. In the assays of table 2 and 3 ovarian weights averaging

TABLE 2

Preparation	Lot no. and dating	Dose R.U.	Test date	No. rats	% in estrus	% with corpora lutea	Uterine av. wt. mg.	Ovarian av. wt. mg.
Antuitrin-S	3194590 3-22-38	1	6-21	11	82	82	107	11.6
	3199126 5-5-38	1	6-28	9	100	100	114	14.2
Follutein	63637 4-4-39	1	6-28	10	100	90	124	13.7
	64456[1] 6-13-39	1	10-31	10	100	80	69	14.1
Antophysin	CN370	1	6-22	10	0	0	43	8.0
	CN407	1	10-31	10	0	0	44	9.4
APL	R7504 11-1-38	1	6-21	10	10	20	72	8.2
	R7842 1-1-39	1	10-31	10	30	10	46	9.5
Pregnyl	—	1	7-26	10	50	30	98	10.6
Untreated Controls	—	—	—	13	—	—	18	10.2

[1] Complimentary sample from manufacturer.

11 mg. or more were usually associated with luteinization and vaginal estrus in a majority of the test rats, though control weights averaged 10.1 mg.

The uterine weight method of Levin and Tyndale[5] and Heller, Lauson, and Sevringhaus[6] for assay of gonadotropic substances is also a sensitive method of assay

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for anterior pituitary-like extracts. In our study uterine weights became maximal before ovarian weights increased appreciably (tables 2 and 3). The uterine weight changes are marked and occur within a narrow dose range. It is a delicate method of assay, especially in the small-dose levels, which give relatively few vaginal estrous and luteinization responses. The sensitivity of the uterus to anterior pituitary-like preparations is illustrated by the difference in uterine weights when the same total amount of material is given by the single-injection and the 2-injection technic. Five

TABLE 3

Preparation	Lot no. and dating	Dose R.U.	Test date	No. rats	% in estrus	% with corpora lutea	Uterine av. wt. mg.	Ovarian av. wt. mg.
Antophysin	CN370	3	6–22	11	100	82	114	12.8
	CN351	2	10–17	11	100	91	85	13.0
APL	R7504 11–1–38	3	6–21	10	70	80	115	13.4
	R7972[1] 1–15–39	2	10–18	9	66	55	80	12.5
	R7842 1–1–39	2	10–31	10	100	80	83	13.1
Pregnyl	—	2	10–18	10	80	70	93	13.0

[1] Complimentary sample from manufacturer.

rats injected once daily had uteri averaging 49 mg. in weight, whereas a similar number injected twice daily had uteri averaging 95 mg. The total dose to each rat, 0.5 R.U. of antuitrin-S, was not sufficient to produce a full vaginal estrus in any of the rats, though a few of those injected twice daily were in proestrum at the end of the test. In the case of pituitary extracts the greater response to the 2-injection technic was also noted by Heller, Lauson, and Sevringhaus.

The uterine weights in the experiment utilizing the single-injection and the 2-injection technic, 49 and 95 mg., contrast with ovarian weights of 9.8 mg. and 9.6 mg., respectively, and emphasize the sensitivity of the uterine weight method as compared with the ovarian weight method at this low-dose level. The ovarian weights at low-dose levels, antophysin and APL 1 R.U. (table 2), appear to be lower than the control ovarian weights. Whether this decrease in weight is real cannot be determined from our few experiments. Nevertheless, it is interesting that Heller, Lauson, and Sevringhaus, using pituitary extracts, and Cartland and Nelson, using pregnant mare's serum, also record ovarian weights lower than their control weights when small doses were administered. A further point of interest in these papers is that ovarian weights increase very definitely before maximal uterine weights occur. In our experience anterior pituitary-like preparations produce a maximal increase in uterine weights with but a slight increase in ovarian weights; these figures illustrate another pharmacological difference between these substances.

The age of a rat conditions its response to pregnancy urine extracts. The age factor is demonstrated by an experiment in which eleven 21-day-old rats and ten 26-day-old rats were treated simultaneously with the same dose, 2 R.U. of antophysin. Thirty-six per cent of the 21-day-old rats, as compared with 88% of the 26-day-old rats, had estrous smears, but luteinization occurred in only 18% of the younger

group and 88% of the older one. Increasing the length of the test period also decreases the potency of the rat unit, since a 120-hour assay with 0.5 cc. R.U. of antuitrin-S produced a vaginal estrus in 50% of the test rats, although all vaginal smears were negative at 96 hours. Thus the difference in the ages of the rats and in the duration of the assay affects the potency of the unit as employed by the manufacturer.

The greater potency of antuitrin-S and follutein may be explained by the fact that they have been standardized on the basis of the luteinization end point, whereas the other preparations are standardized by the vaginal estrous end point, which requires less material and thus is a smaller rat unit. The 120-hour test period employed by the manufacturers in the assay of antophysin and APL tends to decrease the value of the rat unit as compared to that of the 96-hour test periods of the other preparations. However, APL is standardized on 21-day-old rats with single daily injections, a technic which would make a quite potent unit were it not offset by the adoption of the Collip day unit, by which the manufacturer designates as a rat unit only one third of the amount given to the rat over a period of 3 days. For this reason the potency of the unit is reduced, so that it approaches a value similar to that of antophysin and pregnyl.

The causes of the differences in potency of commercial extracts are well summarized by one pharmaceutical laboratory.

> In view of these facts—that different laboratories use rats of different ages, take different end points of assay, and express rat units in different terms of the total amount of hormone used in assay—direct comparison of products on a 'unit for unit' basis is impossible.[7]

Therefore the adoption of a common method of standardization or, even better, of a standard reference powder, as has been done for posterior pituitary preparations, would aid in producing commercial anterior pituitary-like extracts of more uniform biological potency.

Summary. Five commercial anterior pituitary-like products were assayed by a common method to determine their comparative potency. Differences in potency were demonstrated on products all of which were labeled as containing '100 rat units per cubic centimeter.' Two preparations, antuitrin-S and follutein, are approximately twice as potent as antophysin, APL, and pregynl. Some of the factors determining the value of the rat unit were discussed. The adoption of a standard method of assay for all commercial preparations would result in more uniform potency and would aid in determining optimum clinical doses.

The report of the Third International Conference on the Standardization of Hormones[8] appeared after this manuscript was submitted for publication. This report indicates that an international standard preparation of the gonadotropic substance of urine of pregnancy may soon be available.

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[7] Parke-Davis Laboratories, Clinical Progress *Bulletin* No. 17, May 25, 1938.

[8] League of Nations, *Bulletin* of the Health Organization, Biological Standardization III, Volume VII, No. 5, October 1938.

INFLUENCE OF THYROID HORMONE ON SERUM PHOSPHATASE

NATHAN B. TALBOT*

THE LEVEL of phosphatase in the serum is believed to be an index of osteoblastic activity and as such is related to bone formation.[1,2,3] Hypothyroidism during childhood results in retarded osseous development and dwarfing of stature.[4] Thyroid therapy tends to repair this retardation by stimulating bone growth and development. Therefore it is to be expected that the level of phosphatase in the serum will be low in untreated juvenile hypothyroidism and that it will rise following the administration of thyroid. Bodansky and Jaffe,[3] in one cretin girl 4 years old, found the phosphatase to be normal before treatment and to be increased after thyroid therapy. Morris and Peden,[5] however, failed to observe any change over a period of four weeks in two infant cretins who had received thyroid treatment.

TABLE 1. PHOSPHATASE ACTIVITY BEFORE AND AFTER THYROID THERAPY

Case	Comment	Age	Phosphatase, Bodansky units	Increase over control %
B.A.G	Before ℞	4 yr.	3.5	
	5 mo. ℞	2 mo.	10.3	195
	6 mo. ℞		9.1	160
N.W.	Before ℞	3½ yr.	2.7	
	1 week ℞		2.9	7
	3 weeks ℞		4.2	55
	7 mo. ℞		4.7	74
B.B.	Before ℞	10 yr.	5.3	
	5 weeks ℞		7.7	45
	2 mo. ℞		7.4	40
N.N.	Before ℞	1 hr.	1.3	
	1 week ℞	3 mo.	3.7	185
	7 weeks ℞		3.3	154
	3 mo. ℞		18.7	1340
E.C.†	Before ℞	10 yr.	2.2	

† Unfortunately no phosphatase determinations could be obtained on this patient after treatment was started.

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[1] ROBISON, R., AND K. M. SOAMES: *Biochem. J.* 18: 740. 1924.
[2] KAY, H. D.: *Physiol. Rev.* 12: 384. 1932.
[3] BODANSKY, A., AND H. V. JAFFE: *Arch. Int. Med.* 54: 88. 1934.
[4] MEANS, J. H.: Thyroid and Its Diseases. Lippincott, Philadelphia, 1937.
[5] MORRIS, N., and O. D. PEDEN: *Quart. J. Med.* 6: 211. 1937.

According to Bodansky[3] the normal values for serum phosphatase during childhood range between 5.0 and 14.0 units. Table 1 gives the serum phosphatase values of hypothyroid children before and after thyroid treatment. The table shows that the phosphatase was abnormally low before treatment in 4 of the 5 patients. In every instance, the phosphatase rose after treatment was started. There was a significant rise in the phosphatase activity of 2 patients (*N.W.* and *N.N.*) after only 3 weeks of therapy. In 8 other patients whose phosphatase was determined only after prolonged thyroid administration, the average phosphatase was 7.8 and the range 4.4 to 12.0 units. These findings suggest that there is a correlation between the rate of bone growth and the level of phosphatase in the serum of hypothyroid children.

Summary. The serum phosphatase of untreated hypothyroid children tends to be abnormally low (average 3.0 Bodansky units). It is restored to normal levels (average 8.5 Bodansky units) by thyroid therapy.

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ADVANCE ABSTRACTS

Beilly, J. S. Determination of the pH of the vaginal fluid as an objective method of observation in administration of estrogens. *To appear in this Journal.*

A simple test is offered which can be employed as a guide in treatment with estrogens in human castrate, menopausal and hypo-ovarian female groups by following the changes in pH of the vaginal contents. This determination is carried out by adding to the sample of the vaginal contents the universal indicator, and the pH is detected by a color change. Objective and subjective improvement, following administration of adequate amounts of estrogens, is predictable and will be noted with approach of the normal, acid phase of contents. The change in pH occurs concomitantly with improvement in cytology of the vaginal smear, after administration of estrogens. The pH method can be used as an office procedure, and is a guide in estrogenic medication.

Beilly, J. S. Hydrogen ion concentration changes in the vaginal fluid of the rat, during an estrus cycle. *To appear in this Journal.*

Hydrogen ion determinations of the vaginal contents, carried out concomitantly with vaginal smear examination, in normal rats indicate that there is a changing pH which is cyclical in occurrence, and seems related to the cyclical changes in cytology, during an estrus cycle. The alkaline phase is associated with appearance of leucocytes, and the acid phase with 'epithelization' and appearance of estrus. The castrates do not exhibit a changing pH and remain in the alkaline phase. The cyclical nature of the pH can be induced upon return of cycle, after administration of estrogens.

Cameron, D. E., and E. M. Jellinek. Physiological studies in insulin treatment of acute schizophrenia; pulse rate and blood pressure. *To appear in this Journal.*

Insulin treatment was carried out on 22 patients suffering from recent schizophrenia. Pulse rates and blood pressure readings were recorded before and after treatment. The 'recovered' patients showed before treatment greater pulse variability than normal, but less than the 'not-recovered' patients. After treatment the variability of the 'recovered' patients returned to normal limits, while the 'not-recovered' showed little change. The average pulse rate of the 'recovered' patients increased significantly to beyond the normal level, and that of the 'not-recovered' patients remained at normal level. Before treatment the 'recovered' group showed a tendency towards hypotension. The systolic-diastolic correlation was high in both groups. After treatment the 'recovered' group became approximately normal in all recorded aspects of the blood pressure, while little or no change was noted in the 'not-recovered' group. Evidence is presented which suggests that prior to treatment there is some degree of adrenal-sympathetic impairment and that this is largely overcome by treatment in the 'recovered' patients and relatively unaffected in the 'not-recovered' patients.

Cutting, W. C. Changes in growth and function of the thyroid after thyrotropic stimulation. *To appear in this Journal.*

The administration of thyrotropic hormone to guinea pigs causes continued increase of thyroid function as indicated by metabolic and histological changes, until refractoriness is induced. However,

growth of the thyroid gland, as shown by the presence of increased numbers of mitoses, demonstrated by histological examination after the injection of colchicine, is significantly increased only briefly during an early period of the hyperthyroidism.

EVANS, H. E., M. E. SIMPSON AND R. I. PENCHARZ. Relation between the growth promoting effects of the pituitary and the thyroid hormone. *To appear in this Journal.*

Growth promotion in excess of normal (gigantism) secured by anterior pituitary extracts is not dependent on the presence of the thyroid but is greater when the thyroid is present. The growth promotion of thyroidectomized animals or thyroidectomized-hypophysectomized animals in excess of normal (gigantism) secured by anterior pituitary extracts is also maximal only if thyroxin is concordantly administered. Thyroxin, which promotes the growth of thyroidectomized animals, does not have this effect when administered to thyroidectomized-hypophysectomized animals. Characteristic ovarian changes are found in thyroidectomy of long standing. These consist essentially in the substitution of medullary for cortical activity, a growth of the so-called medullary or hilum tubules occurring; in extreme cases the latter structures dominate the ovary, ovarian follicles, corpora lutea and normal interstitial tissue being absent. The administration of thyroxin to such thyroidectomized females prevents this development of the hilum tubules, but anterior pituitary extracts do not have this effect.

EVERSOLE, W. J. The effect of androgens upon the fish (*Lebistes reticulatus*). *To appear in this Journal.*

One hundred fish (*Lebistes reticulatus*) were treated with testosterone propionate for periods varying from birth to maturity. Fish were kept in 2.5-gallon aquaria in groups of from 4 (if adults) to 16 (young broods). Two-thirds of the fish were injected twice weekly, by means of a micro-injection apparatus, with approximately 0.03 mg. of testosterone propionate over a period ranging from 1 week to 4 months. One-third were hormone-fed by adding testosterone in ether solution to the food and then evaporating the mass to dryness. Feeding was done in such amounts that 0.5 mg. was added daily to each aquarium for periods varying from 2 to 3.5 months. Female fish fed or injected with the hormone readily assumed some but not all of the male secondary sex characters. Testosterone administration induced gonopod (male intromittant organ) formation in females at any stage of development but did not induce the typical male red, yellow and orange body pigment. Growth in the female was inhibited (males are normally much smaller than females) but the gravid spot, a distinctive female trait, could not be suppressed. As a result of hormone treatment the gonads of both sexes were markedly reduced in size showing inhibited gametogenic activity and a secondary invasion of lymphoid-like tissue.

FRIEDGOOD, H. B. Adrenalectomy and coitus-induced ovulation in the rabbit and cat. *To appear in this Journal.*

The ovaries of the estrous cat and rabbit respond with ovulation to the stimulus of coitus, although they do not react similarly if the animals are subjected to a laparotomy soon after mating. The rabbit ovulates in spite of major abdominal operations, whereas the cat usually does not. Adrenalectomy usually does not interfere with the occurrence of post-coital ovulation in the rabbit, but ovulation does not ordinarily occur in the cat under similar circumstances. Because of the large percentage of cats which do not ovulate after non-specific abdominal operations, it is probable that adrenalectomy per se also does not influence the progress of post-coital ovulation in the estrous cat.

HART, J. F., AND J. R. LISA. Pituitary cachexia. *To appear in this Journal.*

A case of pituitary cachexia confirmed at autopsy is presented. The patient, a male 37 years old, of the Loraine type of infantilism, was able to carry on comfortably until the last two months of his life. The autopsy disclosed a massive cyst of the pituitary, probably of the pars intermedia, that occupied the pituitary fossa and eroded into the sphenoid. By a stalk it was connected with a large cyst of the right and with a lesser one of the left frontal lobe. The lack of neighborhood symptoms together with the incorrect vizualization of the clinoid processes by the x-ray was remarkable in such an extensive structural change. The histology of the thyroid gland showed complete inactivity and of the testes a marked underdevelopment with only a very early phase of spermatogenesis.

JELLINEK, E. M. Physiological studies in insulin treatment of acute schizophrenia; methods. *To appear in this Journal.*

This paper describes the patient sample and experimental methods used in an investigation of the physiological effects of insulin treatment of 22 patients suffering from acute schizophrenia. The value of small samples is discussed as well as the statistical methods used in the analysis of the data.

LOONEY, J. M., E. M. JELLINEK AND C. G. DYER. Physiological studies in insulin treatment of acute schizophrenia; the blood minerals. *To appear in this Journal.*

Potassium, calcium and phosphorus of the blood were determined on 22 schizophrenic patients 4

times in the 2 weeks preceding and 4 times in the 2 weeks following insulin treatment. Potassium and calcium showed no changes following medication. The mean level of the inorganic phosphorus of the blood was initially significantly higher than in normal controls. The phosphorus level of the 20 patients who recovered dropped significantly after insulin treatment to the normal level. The 'not-recovered' patients, however, showed an insignificant decrease only.

PHELPS, D., E. T. ELLISON AND J. C. BURCH. Survival, structure and function of pituitary grafts in untreated rats and in rats injected with estrogen. *To appear in this Journal.*

In 46 experiments, intact pituitaries from mature male or female donors were grafted into the thigh muscles of totally or partially hypophysectomized mature female rats. Twenty-seven of the hosts were injected during the experiments with 200 rat units of estrogen daily; 19 were not injected. The hosts were autopsied at intervals varying from 5 to 60 days after the implantation of the grafts. Estrogen was found to exert a favorable influence upon the survival of the grafts. Grafts from female donors survived better than did those from male donors, regardless of the treatment of the hosts. All three lobes could be identified in surviving grafts. The anterior lobe was observed to undergo partial degeneration followed by regeneration; these changes were not dependent upon the presence of injected estrogen. Grafts which had undergone regeneration were structurally normal. Those from injected hosts showed the histologic picture which is characteristic of the pituitary response to estrogen; those from uninjected hosts showed a relatively small percentage of chromophiles. Findings relating to the functioning of the grafts were indicative of some, but comparatively little, activity. The ovaries of hypophysectomized rats with surviving grafts showed evidence of mild stimulation but there was no evidence that ovulation occurred or that corpora lutea were formed in these animals. In the injected animals of this group the corpora lutea present at the time of operation underwent regression within 10 days after the implantation of the grafts. There was evidence of mild follicle growth in the ovaries of the rats (of this group) injected for 12 to 18 days.

RANDALL, L. O., AND E. M. JELLINEK. Physiological studies in insulin treatment of acute schizophrenia; the serum lipids. *To appear in this Journal.*

Twenty-two schizophrenic patients in the acute stage were tested for serum lipids (phospholipids, cholesterol, free cholesterol, cholesterol ester and total lipids) in the 2 weeks preceding and in the 2 weeks following insulin treatment. All the mean lipid values, except free cholesterol, were initially significantly lower than the values obtained in normal controls. Following medication, the levels of all the lipids, except free cholesterol, were increased significantly, reaching the normal level in the 'recovered' as well as in the 'not-recovered' patients. Differences between these two types of patients were found in the behavior of the intra-individual variation. In the 'recovered' patients, the intra-individual variation was greatly lowered after treatment, indicating that the lipid regulating mechanisms became stabilized. In the 'not-recovered' patients, however, the chemical action of insulin in raising the lipid levels was achieved only at the expense of increased instability of the regulating mechanism. The increase in lipid values was maintained by the 'recovered' patients during the two weeks following termination of insulin treatment, while the 'not-recovered' patients tended to drop back towards the initial level. Initially, the ratio of cholesterol ester to free cholesterol showed great inconsistency in the subsequently recovered patients, but normal consistency in the 'not-recovered' patients. This initial anomaly in the subsequently recovered patients forms a syndrome with other anomalies, such as hypotension and undernutrition, and it is suggested that this syndrome forms a suitable basis for the favorable action of insulin. The cholesterol ester-free cholesterol ratio became stabilized in the 'recovered' patients but instability was increased in the 'not-recovered' patients following insulin treatment. Increases in the lipid levels were independent from changes in weight, from the initial lipid levels, and from the magnitude of the insulin dosage.

RANDALL, L. O., AND E. M. JELLINEK. Physiological studies in insulin treatment of acute schizophrenia; The choline esterase activity of the blood serum. *To appear in this Journal.*

The choline esterase activity of the blood serum of 22 schizophrenic patients in the acute stage was determined by the gasometric method of Ammon twice in the 2 weeks preceding and twice in the 2 weeks following insulin treatment. The choline esterase level of the patients was initially somewhat lower than that of the normal controls. This difference, however, by statistical test was not significant. Following insulin treatment the choline esterase level increased significantly irrespective of whether the patients recovered or not. In the 'recovered' patients the intra-individual variation was greatly lessened following treatment, the function becoming stabilized while in the 'not-recovered' patients the intra-individual variation was significantly increased; i.e., the function became highly unstable. In both types of patients the choline esterase level tended in the second week following medication to decrease towards the initial level.

SCHOOLEY, J. P. Technic for hypophysectomy of pigeons. *To appear in this Journal.*

The technic and equipment are described for removing either anterior or posterior lobes, or both, from pigeons. Parenteral anesthetics are superior to respiratory types for bird work. The parapharyngeal approach is employed in this technic in order to avoid injury to the feeding mechanisms and to minimize the chances for infections. The anterior lobe is removed whole and recovered after the operation. Operative mortality is less than 2% and birds have lived one or more years after removal of the anterior lobe. Operations are done at the rate of 5 to 8 per hour.

UOTILA, U. U. The rôle of the cervical sympathetics in the regulation of thyroid and thyrotropic function. *To appear in this Journal.*

One hundred and three experimental and 42 control albino rats were studied to investigate a possible sympathetic secretory innervation of the thyroid and the influence of the cervical sympathetics on the thyrotropic function of the anterior pituitary. The experimental procedures included both unilateral and bilateral sympathectomies, with and without hypophysectomy, with cold as a stimulant of the sympathetic system and of the thyrotropic function of the hypophysis. As an index of thyroid activity, the average cell height was used (cell height index). The anterior pituitary is the principal activator of the thyroid. Hypophysectomy in rats is followed by thyroid atrophy which cannot be compensated by sympathetic stimulation (cold). This indicates that the cold reaction of the thyroid is mediated through the anterior pituitary. Unilateral sympathectomy in the rat neither affects the ipsilateral thyroid lobe nor modifies the histological evidence of thyroid activity after thyrotropic stimulation. The present experiments do not speak for a sympathetic secretory innervation of the thyroid. Bilateral cervical sympathectomy in rats causes a temporary and mild hypoactivity of the thyroid, apparently mediated through the anterior pituitary. Similarly, sympathectomy modifies the cold reaction temporarily. These transient effects of cervical sympathectomy are subsequently compensated through other pathways. The cervical sympathetics appear to be unnecessary for a continued histologically normal thyroid function.

WINDLE, W. F. Induction of mating and ovulation in the cat with pregnancy urine and serum extracts. *To appear in this Journal.*

Pregnancy urine or serum extracts (antuitrin-S and gonadogen) were employed in an attempt to induce estrum, mating and pregnancy in cats. Experiments were performed in 122 animals of which 5 were pregnant at the time and 115 were not pregnant but were judged to be anestrus from vaginal smears. Suitable doses of the two extracts were similarly effectual for follicular growth, ovulation and mating in anestrous and pregnant cats which had not been treated previously with these materials. Induction of estrous vaginal smears, estrous behavior, and the attendant follicular growth, ovulation and pregnancy were more readily accomplished with these substances during the time of year at which cats in captivity enter estrum spontaneously (February to June, inclusive) than during other months. Squamous vaginal smears, with manifestations of heat and with matings, sometimes appeared as results of treatments but without any grossly visible ovarian and endometrial development especially during the anestrous season. Great variation in the effectual dose and no difference between intravenous and subcutaneous treatment were encountered. Hyperovulation and hyperfetation resulted in the instances in which suitable quantities of the materials were injected to bring about satisfactory matings. Retreatment within 2 to 5 weeks after initial doses had been given was frequently ineffectual, the animals apparently becoming refractory in consequence of the earlier injections. Such refractory animals sometimes came into heat spontaneously, mated and became pregnant.

A COMPOSITE ABSTRACT OF SOME ARTICLES ON THE HYPOPHYSIS NOT PREVIOUSLY ABSTRACTED IN THIS JOURNAL

THE DIAGNOSIS and differentiation of hypophyseal tumors from other intracranial tumors by means of the x-ray is discussed by Schwartz (1). The proportion of hypophyseal tumors is given as about 20% of all intracranial tumors and of these chromophobe adenomas constituted about 70%. Changes in the skull found in acromegaly are described. Craniopharyngiomas are more rare and are usually found in children or young people. They tend to raise intracranial pressure and often show areas of calcification. A detailed table of points of differential diagnosis of hypophyseal and other intracranial tumors is given; also an extensive bibliography of hypophyseal tumors.

Trumble describes the symptoms, diagnosis and surgical treatment of tumors arising

near the optic chiasm (2). A short history and the results of treatment in 6 cases of acromegaly, 9 cases of adenoma without hyperpituitarism, 2 tumors of Rathke's pouch and 3 unclassified tumors are given.

The pathological and histological findings in each of the tumors described in the foregoing article and those of 3 additional cases are presented (3). Altogether the findings in 23 cases of tumor in the pituitary region are described.

A comparison of the results obtained with surgery, surgery plus irradiation and irradiation alone in a group of 81 cases of chromophobe adenoma is presented (4). The method of giving the x-ray treatment is described. Patients with a sinusoidal type of tumor showed a better response to x-ray therapy than those with diffuse tumors. Cystic tumors were especially resistant.

In patients with tumors involving the optic chiasm with changes in the field of vision, optic atrophy was present in 8 out of 9 in whom the tumor lay in front of the optic chiasm (5). It was absent in all 5 in whom the tumor lay behind the chiasm. It is suggested that the absence of optic atrophy may be a diagnostic point in localizing tumors in this region.

A lesion of the pituitary believed to be a cholesteatoma is reported in a man of 49 years (6). He had had a long standing infection of the ear with discharge. A mastoid operation relieved some of his symptoms, as vertigo and vomiting, but deficiency of thyroid secretion amounting almost to myxedema and hypogenitalism persisted. There was slight enlargement of the sella turcica and a slight diminution of the fields of vision.

A chromophobe adenoma of the pituitary in a man of 45 years with occasional attacks of 'tiredness' in the left arm and leg and increased tendon reflexes apparently due to pressure on the right cerebral peduncle in addition to symptoms pointing to involvment of the optic chiasm is reported (7). Detailed x-ray studies were made. The symptoms were relieved by operation and postoperative irradiation.

A very large craniopharyngioma with an oblique transfrontal extension causing a difference in the appearance of the papilla of the eye on each side is reported in a woman of 46 years (8). There was also evidence of compression of the pyramidal tracts. The sella was enlarged and eroded. A detailed study including ventriculograms was carried out preoperatively to localize the tumor.

The results of treatment especially by x-ray in 53 cases of acromegaly are discussed (9). The symptoms, laboratory findings, results of physical examination, and x-ray findings in acromegaly are described. No changes in the sella turcica were seen in about one-fifth of the cases. It is recommended that the effect of x-ray treatment be tried before surgery in all cases unless permanent damage to the optic nerves may result from further delay. The results of various methods of treatment are presented in table form.

Two cases of acromegaly are reported (10). One in a woman with onset of symptoms at about 21 years of age with enlargement of hands and feet, enlargement and forward projection of lower jaw, enlargement of tongue, amenorrhea and headache. Treatment of the pituitary with radon seeds produced very good results and later a piece of her jaw and tongue were resected. The second case was a man with onset of symptoms at about 17 years of age with a prolonged course and sudden death at 39 years of age. The brain, skull and pituitary were very greatly enlarged, also the long bones, with osteoarthritis at the knee and hip joints. A marked cervicodorsal kyphosis was present, also glycosuria. Enlargement of the bones, hypertrophy of the tongue and changes in the spine are given as the three most common findings in acromegaly in the order of their occurrence.

A very brief report of a case of acromegaly in a man 28 years old is given (11). The eye changes are especially discussed and the effect on the light reflex in this case compared with that in retrobulbar neuritis.

In acromegaly the output of creatine and creatinine in the urine was found to be fluctuating and to exceed the normal amount (12). In 4 patients with acromegaly, a short history of each of whom is given, the injection of large amounts of estradiol benzoate daily decreased the excretion of creatinine to normal levels. In the one case tested very large doses of testosterone produced a similar effect.

A case of acromegaly in a woman 48 years old is described (13). The onset of the condition was probably during her early twenties. At 28 her feet and jaw were enlarged and in

this case there was an abnormal growth of facial hair. When she was 43 the sella turcica was seen to be enlarged by x-ray and irradiation given. The next year her sight began to fail. In the next few years her thyroid showed hypertrophy. At the time of her examination she had been blind for one year and showed a typical picture of acromegaly plus hirsutism.

A case of acromegaly with sexual perversion is reported (14). He apparently had little or no sexual desire or power left but he frequently visited prostitutes and abused them so that complaint was brought against him. He was married and did not abuse his wife although his mood was unstable.

A case of acromegaly associated with diabetes and xanthoma diabeticorum is described in a young man (15). The case was followed over a period of 4 years during which time he had an elevated blood sugar and increased blood lipoids most of the time in spite of large daily doses of insulin. He did not keep to his diet well and was several times threatened with coma.

A case of periostitis of the long bones showing a peculiar double contour in the x-ray associated with repeated attacks of pulmonary infection and acromegaly is described (16). The patient's hands and feet were enlarged and the B.M.R. was increased. Hypertrophic pulmonary osteo-arthropathy has been described by Pierre Marie. Other causes of enveloping periostitis are mentioned.

Two cases of hypophyseal cyst producing Simmonds's disease are reported, one in a woman 59 years old, the other in a male idiot 17 years old (17). The symptoms, pathology and differential diagnosis of Simmonds's disease are discussed. The first patient showed extreme emaciation, marked anaemia, and at one time, hallucinations and confusion. The second patient also showed extreme emaciation. Both patients were autopsied. The gonads, adrenals and thyroids especially showed atrophy and fibrosis. The islands of Langerhans were apparently increased in both patients.

A cholesteatomatous cyst of the pituitary producing symptoms of Simmonds's disease in a boy 13 years old is reported (18). At 7 years of age he had symptoms of diabetes insipidus which lasted for 3 years. After this he began to loose weight and strength. This increased with headache and vomiting. An enlargement of the sella turcica was seen by x-ray. A cystic tumor containing crystals of fatty acid and apparently originating in the pituitary was found at operation. The patient made a good recovery with relief of symptoms.

A glioblastoma in adjacent tissue causing pituitary cachexia in a young man 22 years old is reported (19). There was no actual invasion of the gland by the tumor cells but there were microscopic changes probably due to pressure and irritation. The patient showed increased thirst and polyuria, headache, blurring of vision, loss of weight, atrophy of the testes and finally asthenia so severe he needed assistance in moving in bed. Treatment with gonadotropic hormone from pregnancy urine produced definite improvement for about one month. After this the symptoms gradually increased and the patient died. An autopsy report is given showing atrophy in the other glands of internal secretion including the pancreas.

Two cases of hypophyseal tumor with signs of hypopituitarism are reported (20). The presence of hyperchromatic anaemia with changes in deep sensation, vibratory sense and tendon reflexes, and absence of gastric acidity in one of these cases and loss of the patella reflex in the other is brought out. References in the literature to the association of achlorhydria and changes in the spinal cord with both pernicious anaemia and pituitary insufficiency are given.

A case of Cushing's syndrome is reported in a boy 18 years of age (21). He presented typical symptoms with adiposity of the face and abdomen, striations of the skin, hypertension and induced hyperglycemia. X-ray treatment of the pituitary region produced excellent results.

A case of Cushing's disease associated with severe diabetes is reported in a woman 41 years old (22). She was very obese with a large neck, marked hirsutism, amenorrhea and asthenia. Her blood sugar was increased and the urine showed sugar, acetone and albumen with rare red blood cells and casts. With insulin and other treatment for her diabetes she lived for two weeks but during this time blood nitrogen and the casts and red blood cells in the urine increased and she died of uremia. Autopsy revealed a glomerular nephritis. Both adrenals were enlarged and contained adenomas, the ovaries were sclerosed, the pancreas was

infiltrated with fat. A basophil tumor was present in the anterior pituitary. The suggestion is made that the patient's symptoms of diabetes, amenorrhea and hirsuitism were due to the presumably secondary changes in the pancreas, ovaries, and adrenals.

A condition believed to be dystrophia adiposo-genitalis is reported in a male child 2 years old (23). He was very much over weight and showed under development of the genitals. This was associated with increased cerebrospinal pressure and hydrocephalous. There was no apparent impairment of intelligence. No evidence of tumor in the hypophyseal region was found.

A case with epileptic attacks occurring 2 to 4 times a year associated with adiposity of the trunk, moderate increase of the intake and output of water and increased sugar tolerance is reported (24). X-ray treatment of the pituitary region produced relief of symptoms over a period of 2 years although no evidence of a tumor could be seen.

Two cases of dwarfism occurring in the same family and associated with epileptic attacks are reported (25). There were 3 other normal children in the family. In the case x-rayed the sella turcica was small. The other case could not be so thoroughly studied. The boy who was a little more than 7 years old was 29 inches tall and weighed 18 lbs. Other body measurements are given in detail. His genitals were very poorly developed and his I.Q. was 36%. The girl, 12 years old, was 37½ inches tall and weighed 42 lbs. At birth each child weighed about 7 lbs. and the failure to grow began during the first year.

Two cases of the Laurence-Moon-Biedl syndrome in the same family are reported (26). There were 6 children in the family and 2 other children, now dead, were believed to have had the same syndrome. No mental abnormalities are known to have occurred in any of the relatives as far back as the grandparents. The first patient, a boy of 12, had always been backward mentally. He showed obesity, hypogenitalism, polydactylism with 7 digits on one hand, and retinitis pigmentosa. The other patient, a brother of 9, showed very much the same symptoms with, however, only 6 digits on each hand and foot.

Two other cases of the same syndrome in one family are reported (27). The patients were the 8th and 9th children in a family of 11. Two other children had an eye condition, possibly a similar syndrome. Both patients were stout, showed mental deficiency and pigmentation of the retina. Buds or x-ray evidence of the beginning of extra digits were seen in both cases. The boy showed hypogenitalism. A very interesting observation on defects of the vessels in the hypothalamus found at autopsy in 2 cases of this syndrome is mentioned.

Another case of this syndrome is reported in a young man, 26 years old (28). He was born with 6 digits on each hand and foot. At 2 years he began to show impairment of vision. At puberty he was obese with hypogenitalism. At 26 he showed pigmentary retinitis, hypogenitalism, marked obesity and childishness with a mentality of about 10 years.

A detailed autopsy report is given of a case of Laurence-Moon-Biedl syndrome occurring in a girl of 7 who was previously reported clinically (29). The genital organs were poorly developed, the kidneys showed abnormalities of development and the thymic tissue was fatty and fibrotic. A detailed description of the pituitary which was small is given. Serial sections of the hypothalamic region were examined and described. The frontal lobes of the brain were small.

Because of the scarcity of mitotic figures even in rapidly growing tissue a method which will arrest their development at a given stage gives a method of measuring the rate of growth (30). Colchicine produces this effect. The result of injection of prolactin on the crop of the pigeon was studied by this method.

The blood vessels of the hypophysis of the monkey were dissected out under the microscope after injection with India ink (31). Their course and distribution is described. The conclusion is reached that the vascular supply between the hypophysis and the hypothalamus in the monkey is too slight to indicate that secretion is regularly carried that way.

The nerves to the hypophysis were studied in detail in the cat (32). The nerves to the anterior lobe were found to come chiefly from the carotid plexus and those to the posterior lobe from the supraoptic tract although each supplied some fibers to other parts of the gland. The possibility that the glossopalatine nerve also supplies some fibers to the anterior lobe is considered. Their terminations in relation to the cells are described.

A detailed report of the structure of the pituitary gland in the opossum is made (33).

The pars tuberalis and intermedia were found to be poorly developed in the adult. The acidophile cells were grouped in a different portion of the gland from the basophile and chromophobe cells.

A detailed description of the pituitary of the lizard is reported (34). No pars tuberalis was found. There is a diverticulum extending from the third ventricle into the pars nervosa. The pars intermedia has a greater volume than the anterior lobe. A description of the type of cells found in the gland is given.

A detailed study of the capillary blood supply of the pituitary of the frog is reported (35). The capillaries were wide in all parts of the gland but especially in the pars nervosa and intermedia. The length of vessels and their surface area per unit volume of tissue was measured and compared for the various lobes. The pars nervosa and intermedia were much more vascular in terms of surface area than in the cat, while the anterior lobe was less vascular. The relative capillary vascularity of the brain and skeletal muscles were also compared with that of the pituitary.

Two classes of acidophiles have been recognized by special staining in the anterior pituitary of sexually mature female rabbits and cats but not in mice or rats (36). The procedure for preparing and staining the tissues is given in detail. Changes in the second type of cell have been found in association with the reproductive cycle. It is very rarely found in the male.

A method of fixing and staining the anterior pituitary of the rat by means of a modified 'Mallory-azan' technic is described (37). This method gave uniform differentiation of the three types of cells.

A modification of Mallory's triple stain is described by means of which the optimum staining with both the acid and base stain can be checked under the microscope (38).

Fixing by a modified Lane's solution and staining with Mallory's connective tissue stain preserved the granular cells of the pars nervosa as in the fresh tissue (39). This also stained the pars anterior and intermedia well.

Invasion of the neurohypophysis by basophile cells derived from the remnants of the pars intermedia is shown to be a normal process in man (40). Five possible sites for the invasion to occur are described. Invasion is more apt to occur in the male. A detailed study of 75 glands is reported.

In individuals with pathological conditions no relation of the amount of basophile invasion of the neurohypophysis to blood pressure level could be established (41). But extensive invasion was found in patients with chronic glomerulo and chronic interstitial nephritis, and also in 7 patients with tuberculosis.

The granulation, percentage of different types of cells and condition of the Golgi apparatus, in the cells of the hypophysis of the female guinea pig at different stages of the reproductive cycle were studied (42). The amount of granulation was found to vary with the stage of the cycle in both acidophile and basophile cells. Two types of acidophile cells are described. The Golgi apparatus was hypertrophied when the cells showed most granulation. A change in the distribution of the mitochondria was correlated with this.

A decrease in the number of eosinophiles and an increase of chromophobe cells in the pituitaries of rats of both sexes over 18 months of age was found as compared with those of rats 3 to 10 months old (43). In old rats with benign mammary tumors there was a still greater tendency for the number of eosinophiles to be reduced. In the pituitaries of old female rats especially, chromophobic and hemorrhagic adenoma were frequently found; also nodules of chromophobic or eosinophilic cells.

Exposure of rats to cold produced a gross enlargement of their pituitaries and increased blood in the gland (44). There was a marked degranulation of the eosinophile cells. Basophile cells contained vacuoles which they discharged. Prolonged exposure to cold resulted in degeneration of many of the cells and the formation of colloid. Colloid tended to accumulate in the pars intermedia and sometimes in the posterior lobe. It is concluded that colloid formation is at least in part a result of cellular degeneration.

Injection of the pituitary of normal rats and of thyroidectomized rats into guinea pigs caused degranulation of the eosinophile cells of their anterior pituitaries (45). Also feeding thyroid gland produced the same results. The possible factors and mode of action are dis-

cussed. The possibility that increased metabolism makes greater demands on these cells with discharge of growth hormone is considered.

Extracts from the blood and urine of patients with retinitis pigmentosa and mental deficiency were found to produce expansion of the melanophores when injected into frogs (46). The degree of the reaction varied at different times with material from the same patient.

The urine of patients with pigmentary cirrhosis of the liver was found to contain a substance which caused expansion of the melanophores of the frog (47). Four patients are briefly described, 2 with diabetes and 2 without. The urine from the 2 more darkly pigmented patients contained more of this substance than that from the other 2 patients. Treating the very pigmented patients with ascorbic acid reduced the amount of pigmentation and the effect of their urine on the frogs.

Urine from pregnant rabbits expanded the melanophores of the frog (48). This effect disappeared 12 hours after hypophysectomy and 34 hours after abortion. Emulsion of the muscle of the pregnant uterus was also found to contain a substance producing the same effect. No other part of the uterus or its contents contained it.

Evidence is presented pointing to the secretion of pitressin and probably pitocin by the pituicytes of the posterior pituitary (49).

A case is presented in which injections of pitressin apparently caused the expulsion of a small stone in the lower end of the common bile duct with emptying of the distended gallbladder and ducts (50).

Of 42 patients with gastric and duodenal ulcers, 18 showed a polyuria (51). The possibility that gastric ulcer is associated with changes in the posterior pituitary is discussed. All of these patients were treated with posterior pituitary preparations with improvement in 40 of them. The most satisfactory method of giving the treatment was found to be by intranasal insufflation of desiccated posterior lobe.

Changes in the gastric mucosa were observed in rabbits after a single large dose of pitressin (52). Areas of hemorrhage and necrosis were found in the fundic end of the stomach after 24 hours. The microscopic appearance of the lesions is described. The experiments were repeated on dogs with similar results.

Gastric and duodenal ulcers were produced in dogs by the injection of pitressin as a result, it is believed, of interference with the blood supply to the tissues by spasm of the muscles and blood vessels (53). The changes were watched under an anaesthetic and pieces of tissue taken for microscopic examination at intervals. Pictures and detailed description of the results are given.

Injection of anterior pituitary extract did not alter the excitability of the sciatic nerve in the frog but posterior lobe extract produced a definite condition of hypoexcitability (54). This did not occur if the nerve was cut centrally to the point of stimulation.

In dogs in which the neck had been crushed so that the head and trunk were connected only by the carotid arteries and jugular veins, stimulation of the central end of the vagus produced a rise in blood pressure believed to be due to secretion of pitressin (55). Ergotoxine or clamping of the adrenal veins did not prevent it. Hypophysectomy or crushing the pituitary pedicle abolished it. A similar type of blood pressure curve was obtained after injections of pitressin.

In hypophysectomized dogs with the neck crushed, as above, treated with eserine, stimulation of the central end of the vagus resulted in a primary depression of blood pressure, followed by a more prolonged elevation (56). In non-eserinized, hypophysectomized animals there was no effect on blood pressure. A larger dose of eserine increased the phase of depression. Atropine abolished it. Ergotoxine prevented the secondary rise of blood pressure. Therefore it is believed that stimulation of the vagus liberates acetylcholine at its central terminations which in turn, by way of the blood stream, stimulates the secretion of adrenalin.

Similar experiments were carried out on cats with the neck crushed (57). The results varied only in that the depressor phase in hypophysectomized, eserinized cats was not obtained. A pressor phase which could be abolished by clamping the adrenal veins occurred however, indicating the secretion of adrenalin. It is suggested that the absence of a depressor phase may be due to a difference in rate of secretion or quantity of acetylcholine. Larger doses of eserine per kg. were required in the cat than in the dog.

In normal cats and those with the neck crushed faradic stimulation of the peripheral

cervical sympathetic nerves produced a hyperglycemia (58). Also stimulation of the afferent vagus caused an increase of blood sugar. Hypophysectomy abolished both these reactions but stimulation of the sciatic nerve was still effective in producing hyperglycemia. It is suggested that the increase of blood sugar after vagal stimulation is the result of an action on the posterior pituitary and that sympathetic stimulation may act through the anterior pituitary. Injection of pitressin increased the blood sugar.

A saline suspension of ox anterior pituitary injected into axolotl in the larval stage resulted in a marked increase in growth and, in all but one, some signs of metamorphosis (59). No further growth occurred after 3 weeks from the time the injections were stopped. Phyone produced increased growth but no tendency to metamorphosis and growth was again established after the injections were stopped. Prolactin caused slight increase of growth. No abnormalities of shape or proportion were seen in any of the animals.

The food intake of axolotls was compared with their increase of weight in normal axolotls and those injected with growth hormone or prolactin (60). Utilization of food was greatest in those injected with a simple saline preparation of the anterior pituitary, and next with the growth hormone.

Complete hypophysectomy in the immature rat resulted in the atrophy of the adrenals reaching a maximum in about 2 weeks and of the testes in about a month (61). There was a slight decrease in the weight of the thyroid in one week. Injury to the floor of the third ventricle caused obesity in half the rats so injured and in the other half it did not. The histological changes found in the testes, adrenals and thyroid at intervals after hypophysectomy are described in detail.

In subjects fed a creatine-free diet the output of creatine was increased by the thyrotropic hormone (62). Anterior pituitary gonadotropic hormone increased the excretion of creatinine. Neither prolan nor growth hormone had any effect. The output of these substances in acromegaly is discussed. The creatinine output was found to be increased in all the cases studied while that of creatine varied.

Extracts of the anterior pituitary caused a definite increase in weight of hypophysectomized rats of the same starting weight (63). A formula was worked out between the increase in weight and the logarithm of the dose used. The error of the method has been calculated.

In 5 subtotally hypophysectomized rats some degree of growth occurred although the pituitary remnants were found to contain only basophile and chromophobe cells (64). The histology of these glands is described in detail. The basophile cells showed changes similar to those seen in castration and in Cushing's disease, believed to indicate excessive secretory activity. In a 6th rat no growth occurred and the pituitary remnant contained only chromophobe cells.

A detailed study was made of the results of testicular homotransplants to the anterior chamber of the eye of the immature rat (65). The temperature of this location was found to approximate scrotal temperature. The survival of grafts here was 91% as compared to 51% at other sites. Grafts in castrate hosts showed greater development of the interstitial tissue than those in normal hosts.

The effect of hypophysectomy on the growth of malignant tumors in male rats was studied (66). In rats receiving the same amount of food growth was much slower in hypophysectomized animals. The tumor growth receded abruptly after hypophysectomy so that the effect was not thought to be due to secondary atrophy of some other gland. Growth in the tumor after hypophysectomy although slow was of the same type as before. Feeding thyroid gland or the injection of adrenal cortical extract did not change the result on the growth of the tumor in hypophysectomized rats materially.

A detailed study of the changes in the anterior pituitary in rats with spontaneous, induced or implanted tumors under various conditions and after the injection of anterior pituitary and ovarian hormones was made (67). Rats with tumors were found to have a reduced number of eosinophiles in their anterior pituitary and an increase of chromophobes to over 70%. Large degranulated cells were also increased in number. It is pointed out that a similar increase in chromophobe cells occurs in normal rats treated with estrogenic hormone. Injection of growth hormone or feeding for long periods with 2-amino-5-azotoluene increased

the percentage of chromophobe cells. Female rats did not produce young after developing tumors. The percentage of the different types of cells in the anterior pituitary was normal in rats found to be immune to tumor growth.

It is stated that adenocarcinoma of the uterus very rarely occurs in the rat (68). Trauma produced this lesion in 1 rat of 30 treated daily with extract of the anterior pituitary of sheep over a long period. Eight developed benign inflammatory hyperplasias but one developed an adenocarcinoma of the endometrium with widespread metastases.

A crude anterior pituitary extract containing growth and gonadotropic hormone was found to stimulate the growth of tumors implanted in white mice (69). Thyroxin under certain conditions retarded their growth. It had no effect on large tumors but in the series tested seemed to have a definite effect when injected in a single dose about a week after implantation.

Biweekly injection of large doses of estradiol caused development of pituitary tumors in infantile mice (70). Arrest of general growth and sexual development occurred first. Tumors could first be recognized macroscopically in the males at about 4 months, those in the females developed later. At 8 months both sexes showed enormous pituitary tumors. These very large tumors showed an amount of gonadotropic hormone similar to that present in a normal gland.

Metastasis of a bronchogenic carcinoma to the pituitary with a resulting polydipsia and polyuria which was relieved by pitressin is reported (71). The sella was seen to be exlarged by x-ray and at autopsy the pituitary was practically replaced by tumor.

The pituitary was removed from tadpoles at the tail bud stage and they were grafted at different stages up to the normal time for metamorphosis with pituitaries from other tadpoles some of them metamorphosized and some not (72). Grafted animals metamorphosized later, more slowly and when they had reached a larger size than normal animals. The stage of development of the donor seemed to make no difference in the rate of metamorphosis. The engrafted glands underwent differentiation at their new site, as indicated by metamorphosis of the host, but more slowly than normally.

Injections of the anterior pituitary gland of frogs stimulated the glandular epithelium of the thyroid in guinea pigs (73). That this was not due to a foreign protein is shown by the fact that injections of brain tissue had no effect.

Rabbits were treated with thyrotropic hormone for 3 to 4 months with little change in the cholesterol found in the aorta (74). But when they were fed cholesterol in addition there was a marked increase of the cholesterol content. It was concluded that atherosclerosis was dependent rather on increased cholesterol in the blood than thyrotropic hormone.

A detailed article on the thyrotropic hormone is presented (75). Four stages of reaction to the thyrotropic hormone are described in the thyroid gland of the guinea pig. Methods of concentrating the amount of thyrotropic hormone in the blood and urine are described. No thyrotropic activity was found in blood or urine from patients with Graves disease with 2 exceptions. Rather the serum had a depressing effect on the thyroid gland believed to be due to an excess of thyroxine. Serum from 2 patients with myxedema was without thyrotropic effect also. Serum and urine from normal animals and man showed no thyrotropic effect on the guinea pigs.

A long detailed review of the recent work of Young and his associates on the relation of the anterior pituitary to the pancreas and diabetes mellitus is presented (76).

A marked, severe glycosuria was produced in 5 dogs and maintained for 7 to 21 days by increasing doses of anterior pituitary extract (77). The dogs remained in good condition and gained weight. No acetonuria was observed. The glycosuria disappeared when the injections were stopped except in 2 dogs in which it persisted 7 to 10 days.

Massive irradiation of the hypophysis in pancreatectomized dogs did not lower the blood sugar, or change the type of response to the ingestion of sugar or increase the effectiveness of insulin or in any way ameliorate the symptoms in the 7 dogs tested (78). The dosage and method of giving the treatment is described. Six of the animals were autopsied and serious damage to the hypophysis found, also definite damage to the cortex of the brain.

The effect of prolactin on carbohydrate metabolism in rabbits was studied (79). The methods of preparing the prolactin fraction used in these experiments is described. Previous injection of prolactin markedly inhibited the action of insulin in reducing the blood sugar

level in adult rabbits. In young rabbits it had very much less effect. Nevertheless it inhibited the formation of liver glycogen. It had no significant effect on glucose tolerance.

The gonadotropic activity of 109 human pituitaries, 52 of which were from patients with intracranial tumors causing increased pressure was tested (80). Gonadotropic activity was found to be more variable in cases with increased pressure but no definite relation could be established. In the controls it was found that gonadotropic activity increased in the females until around the menopause when it showed a sharp rise, and in men to around the 70th year.

The serum of 9 patients under treatment for undescended testicle was examined for antigonadotropic activity after a period of biweekly treatments lasting in some patients as long as 7 months with pregnyl, antuitrin-S or pig anterior pituitary extract (81). None or only a slight degree of activity was found.

Anterior pituitary follicle stimulating hormone was given to a group of 14 women before laparotomy for various conditions and its effect on the ovaries noted (82). All these patients had supposedly normally functioning ovaries. In all but one, to whom only a small dose was given, and another to whom luteinizing hormone was given at the same time with production of 3 recent corpora lutea, an increased follicular growth was found. This occurred even in one case of pregnancy and 3 recently delivered cases. The results were similar to those previously reported in apes.

No increase in the gonadotropic hormone was found in the urine of 28 cases of different types of lesion of the breast with the exception of one of tuberculous mastitis, nor in 32 subjects with enlarged prostates (83). It was present in 2 teratoma testes and one chorionepithelioma.

Evidence that estrin is the active agent in maintaining corpora lutea is presented (84). After hypophysectomy injections of extract of pregnancy urine were ineffective in maintaining corpora lutea but injections of estrin maintained it with progestational type of endometrium for as much as 10 days. It is believed that gonadotropic hormone acts by stimulating the secretion of estrin. Triphenyl ethylene was also effective in maintaining corpora lutea in hypophysectomized rabbits.

A chemical test for prolans in the urine suggested by Knoop's histidine test is described (85). In cases of teratoma testes it was positive in 39 out of 44 cases and in all 11 cases of pregnancy in which it was used. The method of preparing the urine and making the test is described.

Of 37 patients with functional irregularities of menstruation treated with gonadotropic hormone none developed a progestational type of endometrium and in only 6 was there lessened bleeding (86). It was the author's experience that other methods of treatment as rest, oxytocics, heat, iron, etc. were more effective. Many of these patients had, however, been previously treated with gonadotropic hormone before being referrred to the author, which may have influenced their reaction.

Large doses of extract of human pregnancy urine, male urine and serum from pregnant mares caused enlargement and descent of the testes and spermatogenesis in immature hypophysectomized ground squirrels (87). Similar results were obtained with hypophysectomized adults. Secondary sex organs of similar development to those during the breeding season were seen in some.

Castration changes did not appear in the anterior pituitary of rats after removal of the ovaries if the hypophyseal pedicle had first been severed (88). Administration of estrin, however, caused glands similarly treated to enlarge and show typical cellular changes.

The ovaries atrophied in rats after severing of the hypophyseal stalk (89). Nevertheless, administration of prolan was followed by the presence of large follicles and corpora lutea which does not occur after hypophysectomy. This is believed to indicate that while the gonadotropic function of the anterior pituitary is reduced, it is not abolished.

Administration of estrin to young rats caused corpora lutea formation (90). This effect did not occur after hypophysectomy. If estrin was injected 4 days previous to hypophysectomy corpora lutea formation resulted but not if it was injected 3 days before hypophysectomy. If estrin was given 2½ days or more before severing of the hypophyseal stalk luteinization of the ovaries resulted, but not after this time.

Twenty-one positive pregnancy tests were obtained by intradermal injection of antuitrin-S in a group of 26 children, most of them boys (91). Therefore it is suggested that this is not a very reliable test.

Tests for pituitary function in women with menstrual irregularities by means of the glucose tolerance test, B.M.R. and endometrial biopsies are described (92). It is believed that these are the most valuable tests for this type of patient. Tests were carried out on the same day of the menstrual cycle and repeated in subsequent months. Good agreement was found between the glucose tolerance and endometrial findings. If the pituitary was found to be hypofunctioning stimulation was produced by x-ray treatment.

Corpora lutea formation could be induced in some adult cats in estrus by anterior pituitary extract or prolan given intravenously (93). The dose required was very much larger than in rabbits. The secretion from these corpora lutea as indicated by the reaction of uterine muscle to epinephrine and the development of the endometrium, persisted about 20 days. In cats no relation could be established between the reaction of the uterus to posterior pituitary extract and the presence of secreting corpora lutea.

A decrease in the formation of red blood cells in the bone marrow was found in hypophysectomized rats (94). Formation of white blood cells seemed to be normal. Crude hypophyseal extract restored the appearance of the bone marrow to normal. Thyroidectomy did not prevent this action. A thryotropic hormone was no more effective than the crude extract. The fragility of the red blood cells was not found to be increased during one month after hypophysectomy.

A reticulocytopenia was found to develop in hypophysectomized rats (95). Injection of an extract containing both thyrotropic and gonadotropic hormone, growth hormone, an extract of the posterior pituitary containing both hormones, or injections of cortin did not restore the reticulocyte count to normal. Injections of a crude extract of beef anterior pituitaries maintained a normal count.

Implants or injection of anterior pituitary extract caused definite changes in joint and epiphyseal cartilage (96). The effect was studied in detail on young female guinea pigs during the first few days after treatment. Hyperplasia and hypertrophy in the cartilage of the epiphysis and joint usually preceded by degenerative processes in the epiphyseal cartilage are described. No correlation of the changes seen in the cartilage and those in the ovary could be established. Pituitaries from cattle of different ages and sex were used with about the same results.

The presence of thyrotropic hormone in the urine of patients with affective psychoses was investigated by injection in guinea pigs (97). Nine patients with enlargement of the gland or signs of overactivity were selected, of these 6 showed thyrotropic hormone in the urine. The frequency of psychic symptoms in Graves-Basedow's disease is mentioned. Six cases of schizophrenia were examined for the gonadotropic hormone. The pituitary follicle maturing hormone was present in all, but no luteinizing hormone; and folliculin in only 2.

In normal women vasopressin injected intravenously produced a rise of at least 30 mm. in blood pressure and definite subjective and objective symptoms (98). In normal pregnant women after the 6th month there was little or no reaction. The reaction returned to normal soon after delivery. Pre-eclamptic women showed a minimum elevation of blood pressure of 35 mm. with slowing of the pulse and subjective symptoms in 34 observations. The reaction in pregnant women with glomerular nephritis was like that in normal pregnant women.

The amount of vitamin C in the adrenal gland and the anterior lobe of the pituitary after daily injections of nicotine for 15 to 130 days was determined in guinea pigs (99). The amount of vitamin C was increased to the 60th day, after this it decreased. The results in the anterior pituitary ran parallel to those in the adrenal.

A syndrome believed to be due to lowered pituitary function with poor appetite, low blood sugar, respiratory quotients of 0.65 to 0.76, subnormal temperature, low blood pressure, irritability and unstable mood is described (100). That secondary adrenal hypofunction is also a factor is suggested. Simmonds's disease is discussed, especially the failure of the circulation to adjust to muscular effort and the ability of suprarenal extract to raise the respiratory quotient in these cases.

The muscular ability of rats was tested by the length of time they could continue

swimming (101). Hypophysectomy very markedly diminished this time. It was not increased in hypophysectomized rats by adrenal cortical extract, desoxycorticosterone or an increased intake of salt. Neither did injections of anterior pituitary extract increase it.

Galactose or mannose, perhaps both, were found in gonadotropic hormone and prolan, probably in combination with protein (102). Hypophyseal extract and pregnancy urine also contain uncombined sugar which may be used in synthesis of the hormone.

A review of the circulatory and metabolic disturbances related to pathology of the pituitary gland is presented (103).

Rats fed a fat diet showed an increase of glycogen in the liver (104). The excretion of acetone bodies in rats fed on a fat diet was increased immediately after a period of fever. Fever inhibited the development of glycogen in the liver of these rats. Injections of anterior pituitary extract caused a marked increase in excretion of ketone bodies on the days it was injected and prevented the storage of liver glycogen on a fat diet.

Choline chloride was ineffective in preventing the increase of fatty acids in the liver of rabbits after an injection of anterior pituitary extract (105). The iodine numbers were lower in the treated than in untreated animals suggesting that there had been an interference with the desaturation which normally takes place in the liver.—Dorothea Whitney.

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